LEARNING INFANT ASSOCIATED SOUNDS: A BEHAVIORAL PARADIGM TO INVESTIGATE SENSORY PLASTICITY IN A SOCIAL CONTEXT

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LEARNING INFANT ASSOCIATED SOUNDS: A BEHAVIORAL PARADIGM TO INVESTIGATE SENSORY PLASTICITY IN A SOCIAL CONTEXT

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It is not knowledge, but the act of learning, not possession but the act of getting there, which grants the greatest enjoyment

Carl Friedrich Gauss

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SUMMARY

Auditory cortical representations are shaped by diverse and complex experience dependent factors. In the pursuit to understand how these dynamic representations influence social behavior, auditory cortical researchers are turning to ethological paradigms whereby rodents form auditory associations though social interactions. One such ethological paradigm is the maternal mouse communication model, which has been used to investigate plasticity in auditory cortical representations of pup ultrasonic vocalizations. However, questions have recently emerged regarding the degree for which these representations are in fact shaped by social experience. It appears that we now need the ability to "pair" pup interaction with a novel sound that is under experimenter control. Additionally, this method of pairing needs to be done in such a way to allow for the characterization of auditory cortical activity. The development and validation of such a behavioral paradigm is the central aim of this thesis. We have developed a pairing paradigm where we use a novel sound to guide mice to a target arm at the end of a maze where they receive a pup reward. We found that early on in training mice follow a strategy that is not random but is based on returning to the last arm they received a pup. Over training mice shift from using this initial location-based strategy to using an auditory one where they use the delivered sound to seek out and retrieve pups. By silencing auditory cortical activity in mice after they had been conditioned to approach a novel sound for pup reward, we demonstrated that performance on the task significantly drops and that the mice become more likely to use their initial location-based strategy. Finally, we found that mother mice can learn this task faster than cocaring mice. From these results, the paradigm we have developed looks to be a valuable tool for investigating how auditory cortical representations can influence behavior in social contexts.

CHAPTER 1
INTRODUCTION

This chapter was adapted from a previously published book chapter the author wrote reviewing auditory cortical plasticity and the maternal mouse communication model (Dunlap and Liu, 2018).

How stimuli are represented in the brain and translated into physiological and behavioral responses is a fundamental question in neuroscience. For acoustic stimuli, recent anatomical and physiological studies suggest that auditory cortex plays a key role in this transformation, where it goes beyond merely processing sound features, to also being involved in multisensory and even non-sensory processes like memory formation and action selection (Mizrahi, Shalev, and Nelken, 2014; Froemke, 2015; Sutter and Shamma, 2011; Shepard, Kilgard, and Liu, 2013). In studying auditory cortex though, most research has ignored ethological relevance and context (Bennur, Tsunada, Cohen, and Liu, 2013). However, given that the auditory system presumably evolved to deal with ethological sounds in an organism's natural environment, which are often presented in a social context, a deeper understanding of the role of auditory cortical processing and plasticity for driving behavior necessitates investigating how auditory cortex processes sounds during social interactions.

One approach to do this in rodent models is to exploit the social interactions present in maternal behavior, where parental animals form auditory associations for infant rodent vocalizations (Bennur et al., 2013). Although this approach allows investigating auditory cortical coding and plasticity of ethological sounds during natural social behavior, it is lacking a degree of control over the associated sound, which ultimately prevents studying what mechanisms underly the plasticity that occurs in this context. The central aim of this thesis is to develop a behavioral paradigm

that bridges this gap by pairing a sound under fine experimenter control with infant interaction.

In this chapter, we first review recent progress that has been made in our understanding of rodent auditory cortical processing of sound. We present this research along with a contemporary view of a multimodal, dynamic and plastic auditory cortex that empowers salient and meaningful sound categories to drive downstream neural circuits for behavioral output. We will then review brain regions that are traditionally associated with regulating the onset of maternal responsiveness. Next we discuss the maternal mouse communication model as an approach to study the role of auditory cortical processing and plasticity in this context. Finally, we will discuss open questions, which the maternal mouse communication model cannot answer, and which serve as the motivation for our work.

1.1 Auditory cortex

1.1.1 Connectivity of auditory cortex

The representation of socially relevant sounds at the auditory cortical level is first and foremost determined by its pattern of connectivity with the rest of the brain. Below we summarize the general nature of such connections, but deeper anatomical insight can be found in other reviews for rodents (Eike Budinger and Scheich, 2009; Romanski and LeDoux, 1993; Roger and Arnault, 1989) and other mammals (Winer and Schreiner, 2010; C. C. Lee and Winer, 2011; Hackett, 2002).

Auditory cortex receives auditory thalamic input from the medial geniculate nucleus as well as input from other cortical and subcortical regions that provide non-auditory sensory as well as non-sensory information (Eike Budinger and Scheich, 2009). Known sources of non-auditory sensory information include the visual and somatosensory cortices, and multi-sensory posterior parietal cortex (Budinger, Heil, Hess, and Scheich, 2006; Ghazanfar and Schroeder, 2006; Bizley, Nodal, Bajo, Nelken,

and King, 2007; Fu et al., 2003). Non-sensory afferents into auditory cortex also arise from motor cortex (Nelson et al., 2013) and limbic areas such as orbital cortex, cingulate cortex, and the lateral amygdala (Eike Budinger, Laszcz, Lison, Scheich, and Ohl, 2008). Furthermore, the neuromodulators dopamine, serotonin, noradrenaline and acetylcholine, which are thought to mediate arousal, attention, motivation and experience-dependent cortical plasticity, exert further non-sensory influences directly within auditory cortex (Gu, 2002; Stark and Scheich, 1997; Eckenstein and Thoenen, 1983; Manunta and Edeline, 1997).

Such diversity and complexity in the multi-sensory and non-sensory interactions with auditory information which occur in auditory cortex, suggests that it may help build up a multimodal representation of sound shaped by behavioral context (i.e. an auditory object) and used to directly influence behavior. This concept is reinforced by the pattern of outputs from auditory cortex. There are well-described auditory-specific outputs back to the thalamus and subcortical auditory stations (Llano and Sherman, 2008; Budinger, Heil, and Scheich, 2000; Ryugo and Weinberger, 1976; Winer, 2006; Suga and Ma, 2003), which presumably dynamically influence the feedforward processing of sounds. Additionally, there are also direct projections to other sensory, limbic, and associative and/or memory encoding areas, including non-auditory sensory cortex, amygdala, striatum and perirhinal and entorhinal cortex (Znamenskiy and Zador, 2013; Budinger et al., 2006; Eike Budinger et al., 2008; Romanski and LeDoux, 1993). Together, this pattern supports auditory cortical representations modulating acoustic perception, learning and behavioral control.

1.1.2 Representations in auditory cortex

Auditory cortical representations are shaped by context-dependent non-auditory factors

Auditory cortex's diverse connectivity suggests its representation and processing of sounds is shaped by context-dependent, non-auditory factors. Indeed, studies using nonsocial context have demonstrated that passive exposure to visual (Kayser, Petkov, and Logothetis, 2008; Charles E Schroeder and Foxe, 2002) or somatosensory (Fu et al., 2003; Lakatos, Chen, O'Connell, Mills, and Schroeder, 2007; C E Schroeder et al., 2001; Charles E Schroeder and Foxe, 2002) modalities modulates auditory cortical responses to tones or noise. In fact, in ferrets as high as 11-15% of auditory cortical neurons can be either modulated or driven by visual input (Bizley et al., 2007; Mao, Hua, and Pallas, 2011). In social contexts, recent rodent studies affirm the dynamic modulation of USV processing by multimodal stimuli simultaneously emitted by a conspecific vocalizer. For example, adult rats vocalize and whisk in an orchestrated signaling sequence that results in receivers being stimulated simultaneously by both calls and social touch. Auditory cortical neural responses to USVs are strongly modulated in the presence of facial touch (Fig. 1.1) versus without it (Rao, Mielke, Bobrov, and Brecht, 2014). Intriguingly, multimodal stimuli affect neuronal firing differentially depending on cell type, suggesting a locally-targeted rather than nonspecific gain modulation of the auditory circuit.

Auditory cortex is not only affected by external sensory cues from other modalities, but also by internal ones. Movement- (or vocalization-) induced motor signals suppress auditory cortical activities in marmosets (Eliades and Wang, 2003) and rodents, potentially through direct projections from motor cortex (Schneider, Nelson, and Mooney, 2014; Zhou et al., 2014; Nelson et al., 2013), which also target subcortical auditory centers feeding into auditory cortex (Williamson, Hancock, Shinn-

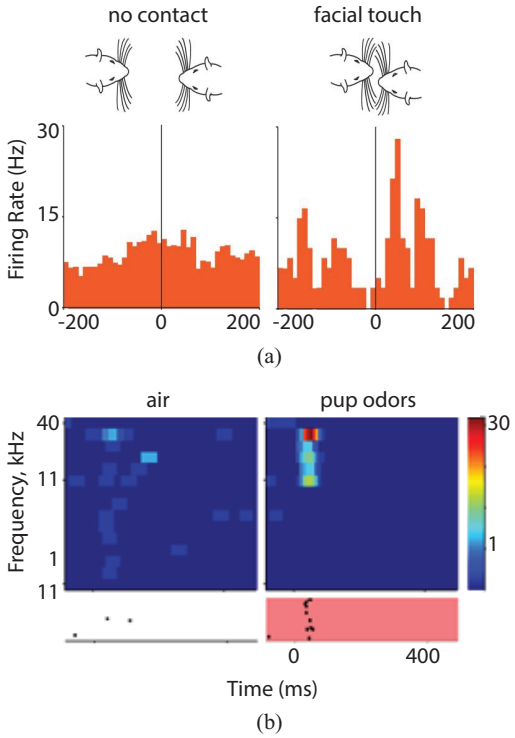


Figure 1.1: Single unit exemplars of multisensory interactions in auditory cortex. **(a)** In rats, the response to USVs for regular-spiking (RS) neurons can be strongly modulated by facial touch. Left side shows peri-stimulus time histogram (PSTH) of RS neuron when facial touch is not occurring and the right side shows PSTH of the same RS neuron during facial touch. Time zero is with respect to USV production. Adapted from Fig. 5 of Rao, Mielke, Bobrov, and Brecht (2014). **(b)** In mice, the tuning of auditory cortical neurons can be modulated by odor. The left side shows the spectro-temporal response field (STRF) of an auditory cortical neuron in an anesthetized lactating mother being modulated by pup odor (right). Adapted from Fig. 1 of L. Cohen, Rothschild, and Mizrahi (2011).

Cunningham, and Polley, 2015). These motor influences may provide an efference copy to the auditory system of self-generated sounds (Schneider and Mooney, 2015), enabling self-monitoring (Eliades and Wang, 2008). Other internal or behavioral state conditions such as arousal, attention, task engagement or hormones also modulate auditory cortex, often on short time scales (Fritz, Shamma, Elhilali, and Klein, 2003; Kato, Gillet, and Isaacson, 2015; Otazu, Tai, Yang, and Zador, 2009; Marlin, Mitre, D'amour, Chao, and Froemke, 2015). Taken together, these studies reinforce the dynamic, context-dependent nature of auditory processing.

Representation of acoustic features of pup cues

Besides the remarkable degree of behavioral state modulation of auditory processing, there are complexities even when animals are in a consistent state. A central objective in auditory neuroscience is to understand these complexities by explaining how sound is transformed into neural responses. Mathematical models that can reproduce and predict neural responses to sound provide a tool to do so. This approach has been successfully applied to the auditory periphery where neural responses can be predicted with high accuracy (Heinz, Bruce, and Carney, 2001). However, higher along the auditory pathway, our ability to model the increasingly complex computations that underlie its neural representations declines. At the level of auditory cortex, no model exists that is capable of fully characterizing how individual neurons are driven by all sounds. For example, standard models used for auditory characterization such as best frequency maps or spectrotemporal receptive fields can depend on the statistics of the sound ensemble used to fit the model (Blake and Merzenich, 2002; Valentine and Eggermont, 2004; Laudanski, Edeline, and Huetz, 2012; Mizrahi et al., 2014).

Despite this, significant progress has been made in understanding sound coding within auditory cortex by asking more targeted questions, where instead of attempting to determine how neurons are driven by all sounds in general, one only considers how

certain features of neural activity are driven by a smaller set of sound classes. With this approach it is possible to use modeling techniques to investigate how features of those sound classes are being represented and used to distinguish stimuli within and across classes. For social sound processing in rodents, this is typically studied using USVs with sound classes that naturally are emitted in various situations, such as male vs. female; adults vs. pups; aggression vs. mating or pup isolation. A key benefit to using rodent USVs, which are mostly single frequency whistles, is their relative simplicity as natural auditory stimuli. This provides practical advantages over vocalizations from other species that have multiple simultaneous frequency components (e.g. harmonics) that can complicate parameterization for neurophysiology studies (DiMattina and Wang, 2006; Kanwal, Matsumura, Ohlemiller, and Suga, 1994).

Exploiting this, a model of pure tone nonlinear amplitude envelope integration at the periphery (Neubauer and Heil, 2008) can be extended to predict the first spike latency in auditory cortical responses to USVs (F G Lin and Liu, 2010). Interestingly, physiologically distinct subclasses of neurons were found to differ in their faithfulness in encoding acoustic features versus behavioral relevance. Specifically, a subset of putative fast-spiking interneurons in mouse core (i.e. primary) auditory cortex have more predictable USV first spike latencies, while a subset of putative pyramidal neurons, whose first spike latencies would not be well predicted by sound envelope integration, appear instead to play a role in differentiating behaviorally significant USV categories (Shepard, Lin, Zhao, Chong, and Liu, 2015). These USV categories are likely being differentiated by their amplitude and frequency modulations, as suggested by the fact that auditory cortical neurons can be sensitive to amplitude and frequency modulations that are more prototypical of natural USVs (Carruthers, Natan, and Geffen, 2013). As discussed further below, the sensitivity to more prototypical modulations can also be shaped by experience and behavioral relevance (Shepard, Liles, Weinshenker, and Liu, 2015).

At the level of the core auditory cortex then, a significant subset of neurons responds most strongly to sound features that are prevalent in natural, behaviorally relevant vocal categories. Additionally, evidence is beginning to accumulate suggesting that neural representations invariant to natural acoustic variability within a vocal category may be built up in secondary auditory cortical areas (Carruthers et al., 2015; Tsunada, Lee, and Cohen, 2011). This could then serve as the foundation for more categorical perception, and drive downstream areas involved in category recognition and behavioral control (Q. Xiong, Znamenskiy, and Zador, 2015; Gifford, MacLean, Hauser, and Cohen, 2005).

Plasticity in auditory cortical representations

How responses along the hierarchical auditory pathway come to be is a separate but also important question. Studies using socially relevant sounds are helping to elucidate the natural mechanisms by which neural responses change due to experience, occasionally in ways that are constrained by evolution, which makes ethological approaches important. Such sound experience can be either passive or active. Passive sound exposure involves simply hearing sounds in the background without correlated behavioral consequences, whereas active experience involves paradigms like classical or operant conditioning, or social engagement, wherein a behaviorally relevant association is made with the sound.

The acoustic environment an animal is raised in during development is essential for shaping their auditory cortical responses to sounds. Studies of the effects of developmental sound experience mostly employ passive exposure rather than active experience. Early life passive sound exposure can alter many physiological properties of auditory cortical neurons, the classical example of which is auditory tonotopic map plasticity (Schreiner and Polley, 2014). This form of plasticity can be driven by rearing mice in an environment where they are continuously exposed to tones of

a given frequency. This leads to a shift in auditory receptive fields characteristic frequencies towards the exposed frequency, thereby increasing the surface area within core auditory cortical fields tuned to that frequency (overrepresentation). This form of developmental plasticity does not, however, happen for all sounds equally and can be driven more strongly when the sounds have more ethological properties, including those such as the natural rate of USV calls within bouts (Kim and Bao, 2009).

A key feature of developmental plasticity is that sound exposure must occur during a narrow window of time known as the critical period, which is 3 days for mice and rat pups (Barkat, Polley, and Hensch, 2011; de Villers-Sidani, Chang, Bao, and Merzenich, 2007). The critical period signifies a temporal window during which the auditory cortex is highly plastic, and the closure of the critical period has been associated with maturation of auditory cortical inhibition (Rice and Van der Loos, 1977; Zheng and Knudsen, 1999). However, the processes that lead to the closure of the critical period are also influenced by the statistical properties of environmental sounds. In fact, exposure to auditory stimuli with low statistical structure as compared to ethological sounds delays the closure of the critical period and impacts the tonotopic representation (de Villers-Sidani, Simpson, Lu, Lin, and Merzenich, 2008; Chang and Merzenich, 2003).

Ultimately, developmental exposure-induced cortical plasticity has important consequences for the representation and perception of sound in the adult animal. It has been argued to yield more categorical representations of natural sound ensembles in auditory cortex, which could provide a foundation for perceptual categories by enhancing discrimination abilities between frequencies at the boundary of exposed sounds (Han, Köver, Insanally, Semerdjian, and Bao, 2007; Bao, Chang, Teng, Heiser, and Merzenich, 2013). Though auditory cortical map expansion has been a dominant way to characterize plasticity, even for adult learning paradigms, its functional role for perception and learning is still debated, as there are conflicting reports concern-

ing the correlation between the size of map expansion and behavioral performance (Bieszczad and Weinberger, 2010; Polley, Steinberg, and Merzenich, 2006; Recanzone, Schreiner, and Merzenich, 1993; Rutkowski and Weinberger, 2005; Brown, Irvine, and Park, 2004; Talwar and Gerstein, 2001). Indeed, recent results raise questions about whether map expansion in adulthood is as persistent as changes created by developmental exposure, or whether it is the final reflection of a learned auditory category (Reed et al., 2011; Michael P Kilgard, 2012).

In adulthood, passive sound exposure can still drive auditory cortical plasticity, though there are important differences. In the context of map plasticity, driving cortical field changes is typically harder and requires longer sound exposure time (Pienkowski and Eggermont, 2009). Additionally, instead of driving map expansion, passive exposure in adulthood results in increased suppression and inhibition as well as decreased excitation (Pienkowski and Eggermont, 2009; Kato et al., 2015). Behaviorally this decrease in excitatory neural response correlates with the animal habituating to the presented sound. In fact, the decrease in excitatory response to a passively presented sound can be reversed if the sound then becomes behaviorally relevant (Kato et al., 2015). This highlights the importance of studying how behavioral engagement can influence auditory cortical plasticity in adulthood, which we discuss below for active auditory learning paradigms.

Active auditory learning paradigms require an animal to learn an auditory task, such as discrimination or detection, in order to receive reward or avoid punishment. How plasticity manifests due to the differential engagement of neuromodulatory systems (Gu, 2002) can depend on the exact nature of how a sound gains behavioral meaning (David, Fritz, and Shamma, 2012). In order to understand the mechanisms of cortical plasticity that drive active auditory learning of socially relevant sounds, we need to study these mechanisms within the appropriate behavioral context by replicating the task/reward structure under which they naturally occur. This mo-

tivates ethological approaches to studying communication sound processing such as the maternal mouse USV communication model discussed below.

1.2 Maternal neural circuitry

1.2.1 Connectivity of maternal neural circuitry

Key nodes in the regulation of parenting are the medial preoptic area (MPOA), bed nucleus of the stria terminalis (BNST), ventral tegmental area (VTA), medial amygdala (MeA), nucleus accumbens (NAcc), and the lateral septum (LS) (Michael Numan, 2007; Kohl et al., 2018; Numan and Sheehan, 1997). Of these, the MPOA is the area most popularly know for its role in the positive control of pup responsiveness. Lesions of the MPOA block pup retrieval and other parental behaviors (Michael Numan, 2007). Evidence suggests that MPOA-lessioned females do not find pups rewarding, implicating the MPOA in the motivational aspects of parental care (A. Lee, Clancy, and Fleming, 2000). The MPOA has reciprocal connections to all of the areas mentioned above as well as other sub-cortical areas (Kohl et al., 2018). A working model of the MPOA presumes that these projections inhibit the MeA, an area associated with pup-directed aggression and avoidance (Numan, Numan, and English, 1993), and excite the VTA to promote parental behavior through the NAcc (Banerjee and Liu, 2013; Michael Numan, 2007; Kohl, Autry, and Dulac, 2017). It is well established that dopamine release from the VTA into the NAcc is involved in motivation, reward learning, and reward seeking (Mirenowicz and Schultz, 1996; Stuber, Roitman, Phillips, Carelli, and Wightman, 2005). How VTA activation could lead to an increase in pup responsiveness during the social context of parenting is not well understood. One hypothesis is that dopamine release from the VTA inhibits NAcc output neurons, which drives behavioral activation through disinhibiting the ventral pallidum (VP). The NAcc sends inhibitory projections to the VP which projects to motor regions and controls behavioral activity (Michael Numan, 2007).

Another central part of the canonical maternal circuit is the BNST. However, activation of neurons within the BNST has been implicated in both pup directed aggression and promoting parental behavior (Tsuneoka et al., 2015; Michael Numan, 2007). As a result, models of maternal circuitry have been proposed that appear to have conflicting roles for the BNST, either inhibiting maternal behavior (Kohl et al., 2017), or promoting it (Banerjee and Liu, 2013; Michael Numan, 2007). It is likely that the BNST plays a role in both, and a more complete picture of its effect on maternal behavior requires delineating between the various nuclei within the BNST. Another area where lesioning disrupts maternal behavior is the LS (Insel, 1986; Wang, Ferris, and De Vries, 1994), which projects to VTA-projecting neurons withing the MPOA (Kohl et al., 2018).

1.3 Maternal hormones

Estrogen, progesterone, and prolactin

Estradiol and progesterone are steroid hormones, which are synthesized and secreted by the corpus luteum in the ovaries (Makris and Ryan, 1975). During pregnancy, levels of both estradiol and progesterone increase and this increase is responsible for many processes that are essential for maintaining the pregnancy. They play critical roles in stopping the estrous cycle, preventing further eggs from maturing, and helping maintain corpus luteum integrity. They prevent uterine contractions and are essential for establishing the placenta. They also interact with prolactin to regulate the development of mammary growth and lactation. Estradiol causes the release of prolactin into the blood and increases the number or prolactin receptors in mammary cells (Tucker, 2000). Prolactin, a peptide hormone which is synthesized and released into the blood stream by the pituitary gland, has been extensively studied with regards its critical role in the initiation of lactation. Without it, estradiol and progesterone do not stimulate mammary growth during pregnancy. After birth

estradiol and progesterone blood concentration levels quickly drop, however prolactin levels remain high in order to maintain milk synthesis. Prolactin levels peak during the time periods between nursing which stimulates milk production.

Oxytocin and vasopressin

Oxytocin is a peptide hormone, which is synthesized in the hypothalamus, where it can be released into the brain or transported to the pituitary gland to be released into the blood stream. During pregnancy, oxytocin levels remain low. A spike in oxytocin levels triggers uterine contractions and induces labor. Out of phase from prolactin, oxytocin is released during nursing where it acts to stimulate milk secretion (Kohl et al., 2017). Vasopressin is another peptide hormone synthesized in the hypothalamus and known for its role in osmoregulation and vasoconstriction. However, its molecular structure is very similar to oxytocin and as a result, in high concentrations it can cause uterine contractions (Chan, Wo, and Manning, 1996). Additionally, it has increased expression similar to oxytocin, before birth and during lactation (Van Tol, Bolwerk, Liu, and Burbach, 1988).

1.4 Maternal behavior

1.4.1 Effect of motherhood on behavior

In addition to the dramatic physical changes within the body that are induced by motherhood, there are also substantial behavioral changes. These behavioral changes play an important role in preparing the mother to care for her young. For example, retrieval behaviors are markedly different between lactating mothers and virgin females in response to novel pups. In rats, virgin females typically avoid pups (A S Fleming and Luebke, 1981), but can become sensitized with prolonged exposure to pup over several days. After this period they will retrieve and perform other maternal behaviors (Rosenblatt, 1967). Lactating mother rats on the other hand are

spontaneously maternal upon parturition.

Wild caught female mice will attack pups until parturition (Dulac, O'Connell, and Wu, 2014). Interestingly, laboratory strains of female virgin mice will show spontaneous maternal behavior when presented with pups, which presumably is the result of selective breeding. However, they show retrieval deficits when compared to lactating mothers when tested in a novel environment (Stolzenberg and Rissman, 2011). It is well known that mice are neophobic, and introducing them to a novel environment would induce stress. The fact that mothers are less affected by this stress with regards to parental care suggests that motherhood can reduce anxiety and fear.

In agreement with this is the observation that in rats, motherhood reduces an auditory driven freezing response when compared to virgins (Hard and Hansen, 1985). Additionally, motherhood is associated with an increase in the willingness to take risks. It has been shown that mother rats will spend more time on the open arms of an elevated plus maze when compared to virgins (Love et al., 2005). Presumably this increase in "risky" behavior promotes exploration improves the mother's willingness to foraging for food. They also show elevated levels of aggression towards intruder males, who would normally attack an kill pups that are not theirs (Consiglio and Lucion, 1996).

Taken together, behavioral changes induced by motherhood, allows mothers to better ensure their young will survive. This is achieved by increasing their desire to care for pup, increasing their willingness to go out and forage in order to support the additional metabolic needs of lactation, and increasing their willingness to fight off potential hostile intruders.

1.4.2 Effect of maternal hormones on behavior

Hormone mediated physical changes in the body happen at the same time as behavioral changes during motherhood, suggesting that maternal hormones may also play a

role in altering behavior. This has been confirmed in numerous studies looking at the effects of these hormones on maternal behavior and the canonical maternal circuit. Estradiol and progesterone levels within the MPOA are elevated during pregnancy due to the fact that they both are capable of crossing the blood-brain barrier (Banks, 2012; Pardridge and Mietus, 1979). Estradiol and progesterone, in the presence of prolactin, prime the female for high levels of maternal responsiveness (Rosenblatt, Mayer, and Giordano, 1988; Bridges, 1984). In the case of estrogen, it has been shown that natural variations in maternal care are associated with differences in the estrogen receptor ERα expression in the MPOA (F. A. Champagne, Weaver, Diorio, Sharma, and Meaney, 2003). ERα is a protein that regulates transcription of target genes (Paterni, Granchi, Katzenellenbogen, and Minutolo, 2014), its presence within the MPOA has been hypothesized as a mechanism for regulating maternal behavior through oxytocin receptor binding within the MPOA (F. A. Champagne et al., 2003).

Receptors for the hormone oxytocin are also expressed in areas that are known to play a role in maternal behavior such as the bed nucleus of the stria terminalis, the MPOA, and the lateral septum (Francis, Champagne, and Meaney, 2000). The expression of oxytocin receptors has been shown to be influenced by estrogen (F. Champagne, Diorio, Sharma, and Meaney, 2001). Oxytocin is released diffusely throughout the brain from the paraventricular nucleus in the hypothalamus. Similar to ERα, changes in brain oxytocin receptor distribution has been associated with the onset of maternal behavior in voles (Insel and Shapiro, 1992) and individual differences in maternal behaviour among rats (Francis et al., 2000). However, ovarian steroids are thought to influence maternal behavior by acting on oxytocin systems in the brain (Cort A Pedersen, 1997), while oxytocin is thought to act more directly to stimulate maternal behavior (Insel, 1986; Michael Numan and Stolzenberg, 2009; C A Pedersen and Prange, 1979).

It was found that unlike oxytocin, vasopressin does not induce maternal behavior

in female rats after intracerebroventricular administration (C A Pedersen and Prange, 1979). However, infusion of antagonists for either oxytocin or vasopressin into the MPOA will block pup retrieval in parturient dams, suggesting that vasopressin may also play a role in regulating the onset of maternal behavior (C A Pedersen, Caldwell, Walker, Ayers, and Mason, 1994). Vasopressin has also been implicated in enhancing maternal behavior in males by acting in the lateral septum (Wang et al., 1994).

1.5 Mouse USV communication model

The maternal mouse model has emerged as a useful system for addressing plasticity in the context of learning social communication sounds. Mice learn to recognize and preferentially approach pup USVs after the experience of caring for pups (Ehret and Haack, 1981; Günter Ehret and Haack, 1982). Natural USVs function as a localization signal (Günter Ehret, 2005) that elicit a search and retrieval by maternally motivated mice for pups that have been displaced from the nest. The recognition of this behavioral meaning can be tested in two-alternative choice tests pitting approach to the playback of natural USVs or USV models against lower frequency, behaviorally neutral sounds. The preference for USVs does not exist for naive mice that have not had pup rearing experience, but can be acquired after approximately 5 days caring for pups, suggesting an experience-dependent learning component to an ethological behavior. Dams begin showing this preference more quickly after birth than virgins with the same period of pup experience (Ehret and Buckenmaier, 1994), and the (presumed) hormonal mechanisms for accelerating such sound category recognition is an emerging area of research in auditory neuroscience.

One advantage of using the maternal mouse USV communication model to study the auditory system is the ability to parameterize calls along simple acoustic dimensions. This has revealed that pup USVs form a cluster around prototypical values in a low dimensional space (Fig. 1.2), despite natural variation across individual calls

(Robert C Liu, Miller, Merzenich, and Schreiner, 2003). That pup calls reliably elicit maternal search and retrieval despite this acoustic variability suggests they could be perceived as an auditory category. In fact, behavioral studies have shown that pup call models are categorically perceived by lactating dams along frequency and duration (Günter Ehret and Haack, 1982). Yet these basic features can be shared with other natural USVs from other behavioral contexts, like those emitted by adult males in the presence of females (Neunuebel, Taylor, Arthur, and Egnor, 2015; Hanson and Hurley, 2012; Grimsley, Monaghan, and Wenstrup, 2011). Nevertheless, USV categories can still be separated by incorporating other, more complex acoustic features such as frequency modulation amplitude (Fig. 1.2c), which tends to be less for pup than adult calls (Robert C Liu et al., 2003; Grimsley et al., 2011). Indeed, mice can learn through operant training to discriminate specific USV exemplars having different frequency modulation (Neilans, Holfoth, Radziwon, Portfors, and Dent, 2014), suggesting that their auditory system can use these features to potentially categorize sounds.

The fact that easily parameterized USVs form behaviorally relevant sound categories provides a convenient starting point to exploit this model to understand how the auditory system comes to functionally detect and discriminate salient sound categories. A working hypothesis has been that the change in behavioral relevance of USVs, e.g. between dams (mostly after a full term of pup experience) and pup-naive virgins, translates into long-term auditory cortical plasticity that improves the ability of new USV exemplars to quickly and robustly drive downstream circuits for behavioral response. Interestingly though, when USVs gain relevance through experience, tonotopic map-level changes do not occur (Shepard, Liles, et al., 2015). This adds fuel to the debate on the function of such areal plasticity in sensory cortex, and highlights the value of studying USVs to reveal how adult auditory cortical plasticity naturally serves to enhance the detection and discrimination of real communication

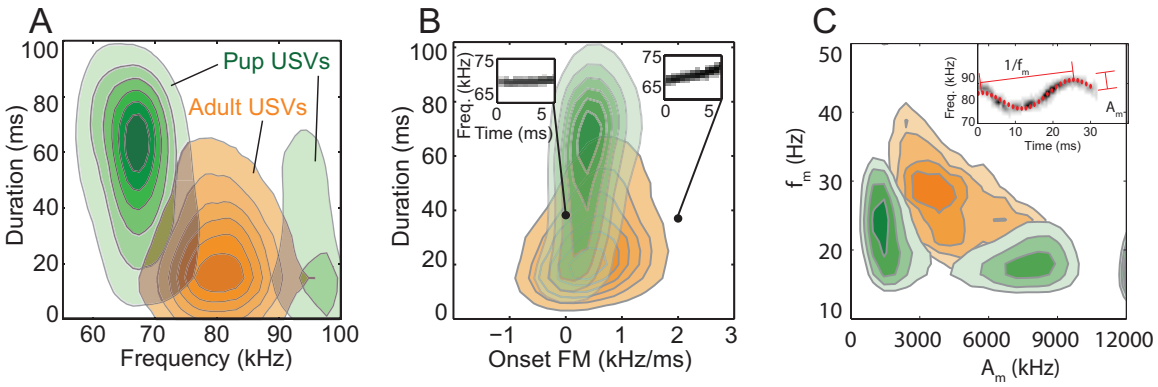


Figure 1.2: Clustering of pup and adult USVs for different features of the calls. **(a)** Green and orange contours represent the distribution of starting frequencies and durations for pup USVs (n = 51,954 USVs, green) and adult USVs (n = 11,248 USVs, orange). **(b)** Contours showing the distribution of onset FM and durations for the same USVs as in panel a. Insets illustrate onset frequency trajectories of two example USVs. Panels a and b adapted from Fig. 1 of Shepard, Lin, Zhao, Chong, and Liu (2015). **(c)** Contours showing the distribution of higher order features derived from the call trajectory for the same USVs as in a, excluding calls that were shorter than 30ms. Frequency amplitude modulation (A_m) and frequency modulation (f_m) were determined by fitting USV trajectories to sinusoidal curves given by $f(t) = f_s + mt + A_m \sin(2\pi f_m t + \phi)$ where the features f_s, m, A_m, f_m and ϕ were computed to minimize sum of squared error. Inset illustrate example sinusoidal curve (red) fit to USV trajectory (black).

sound categories and not just arbitrary synthetic sounds.

Indeed, the lack of map plasticity notwithstanding, several changes in how, rather than how many auditory cortical neurons respond, are observed for both temporal and spectral features of the USVs (Fig. 1.3). For example, consistent with pup isolation USVs being emitted in 5 Hz bouts, multi-unit neural activity recorded from anesthetized mothers is able to more strongly entrain to pup calls presented at this rate over naive animals (Robert C Liu, Linden, and Schreiner, 2006). This form of plasticity indicates that neural activity will be more strongly driven by an entire bout of calls in maternal animals, enhancing their ability to detect the stream. The anesthetized preparation also found improvements in detecting individual USV exemplars (Robert C Liu and Schreiner, 2007), consistent with the idea that motherhood induces long-term changes that enhance functional abilities to process novel examples of the USV category.

Studies in awake animal are beginning to identify distinct coding principles for various subtypes of neural responses, which also serve to enhance the detection of calls. Auditory cortical neurons can be classified as having either an excitatory or a purely inhibitory response to a library of USV exemplars, or having no evoked response relative to spontaneous firing. Interestingly, purely inhibitory USV responses in mothers are stronger and longer than inhibitory responses in naive mice (Galindo-Leon, Lin, and Liu, 2009; F G Lin, Galindo-Leon, Ivanova, Mappus, and Liu, 2013) - an effect that is only significant for neurons at recording locations with best frequencies tuned below typical pup USVs, referred to as a lateral band. Perhaps then the inhibitory response in regions of auditory cortex that should not typically encode calls is stronger in maternal animals to enhance the contrast between USV driven neural activity and neural activity driven by spectrally distinct sound sources. This hypothesized enhanced contrast could improve detection of pup calls by increasing the salience of pup USVs over other sounds in a noisy environment, and also increase

the efficacy of USV-driven activity in auditory cortex to engage downstream brain areas necessary for maternal motivation (Banerjee and Liu, 2013). This interpretation agrees with additional work that found that motherhood increased the best frequency of inhibitory interneurons by a full octave into the USV range, and that no change in frequency tuning was found for excitatory pyramidal neurons (L. Cohen and Mizrahi, 2015). If the shift in best frequencies of interneurons is mainly altering inhibitory response and not excitatory tuning, we would also expect to see enhanced call driven inhibition.

Changes in the auditory cortical representation of USVs also serve to enhance the discrimination of the calls from other sound categories. Building up a more categorical neural representation involves not only tolerance to the natural variability within a sound category, but also enhanced discrimination for sounds outside the category boundary. Such enhanced discrimination is seen for a physiologically distinct subpopulation of neurons in pup-experienced animals. Putative pyramidal neurons with low spontaneous rates and late onset responses in core auditory cortex of maternal mice have excitatory responses that better separate pup isolation USVs from another USV category (adult USVs), whereas this neural separation does not exist for the same subpopulation of neurons in non-maternal animals (Shepard, Lin, et al., 2015). The discrimination of pup calls from other sounds might also be enhanced if other sensory modalities that detect additional pup cues were to modulate auditory responses to USVs. Indeed, since multisensory integration exists in core auditory cortex, the acquisition of maternal behavior could be correlated with plasticity for multisensory integration in auditory cortex. This was indeed discovered for pup odor cues, which modulated both evoked (Fig. 1.1b) and spontaneous activity to enhance USV-detectability in lactating mothers and pup-experienced virgin mice, but not naive virgins (L. Cohen, Rothschild, and Mizrahi, 2011). It is thus conceivable that multisensory representations would further enhance the discrimination of pup calls

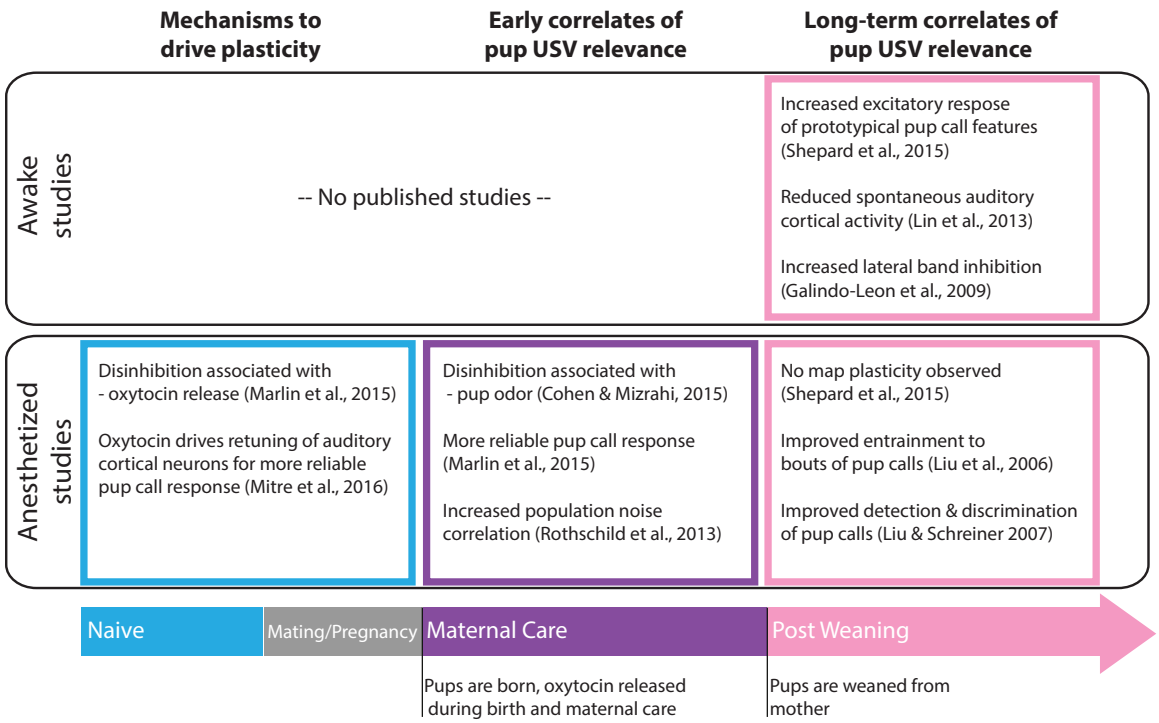


Figure 1.3: Summary of key auditory cortical electrophysiological results using the maternal mouse model. The blue box surrounds results from studies in naive animals, which suggest the sufficiency for oxytocin release during encounters with pups to drive synaptic plasticity in auditory cortex and learn the behavioral relevance of USVs. The purple box surrounds key results from studies done on lactating mothers, typically 4-5 days after giving birth that have found neural correlates of pup call relevance. The pink boxes surround key results from studies done on post weaning mothers within 2 weeks after being weaned that have found long-term neural correlates of pup call relevance. For simplicity, results for pup-experienced virgin mice are not represented but likely undergo oxytocin-mediated experience-dependent plasticity after pup exposure (F G Lin, Galindo-Leon, Ivanova, Mappus, and Liu, 2013; Marlin, Mitre, D'amour, Chao, and Froemke, 2015). Published awake electrophysiological studies of the maternal mouse model used mice that passively listen to sounds rather than those engaging in social behavior. Adapted from Fig. 3 of Dunlap and Liu (2018).

from other USV categories or sounds present in the same context but associated with other multimodal cues that modulate auditory cortical activity in different ways.

1.6 Mechanisms for learning auditory cues in the maternal context

Another advantage of the mouse model is that powerful molecular genetic tools available for mice now provide a strong platform to uncover biological mechanisms for plasticity to enhance the detection and discrimination of new categorical representations. A general framework for conceptualizing such mechanisms (Fig. 1.4) posits that they facilitate immediate changes in the auditory cortical representation of social sound cues during pup experience and initial learning about these sounds, which are then cemented into long-term improvements in their feedforward auditory processing.

One type of mechanism that would act at the time of experience includes disinhibitory circuits engaged by the context. Such circuits are emerging as a potentially universal means for driving dynamic auditory cortical plasticity in social as well as nonsocial contexts (Letzkus et al., 2011). Inhibitory and excitatory synaptic currents onto a neuron are on average largely balanced for pure tones (Wehr and Zador, 2003), though inhibition can be more widely tuned (G. K. Wu, Arbuckle, Liu, Tao, and Zhang, 2008). Spiking is then mainly determined by the relative timing of inputs, whereby excitatory currents typically precede inhibitory currents creating a window on the order of 1-4 milliseconds where the cell is driven to fire (Wehr and Zador, 2003). When inhibitory currents are weakened though, excitation begins to dominate, leading to greater postsynaptic spiking that can induce intracellular cascades promoting the expression of synaptic plasticity genes (Ivanova, Matthews, Gross, Mappus, et al., 2011; Shepherd and Bear, 2011). This process is referred to as disinhibition because it often involves inhibiting GABAergic interneurons that have synapses onto pyramidal neurons, the main output neuron for cortical circuits.

In the maternal paradigm, pup odor increases the spontaneous and evoked activity

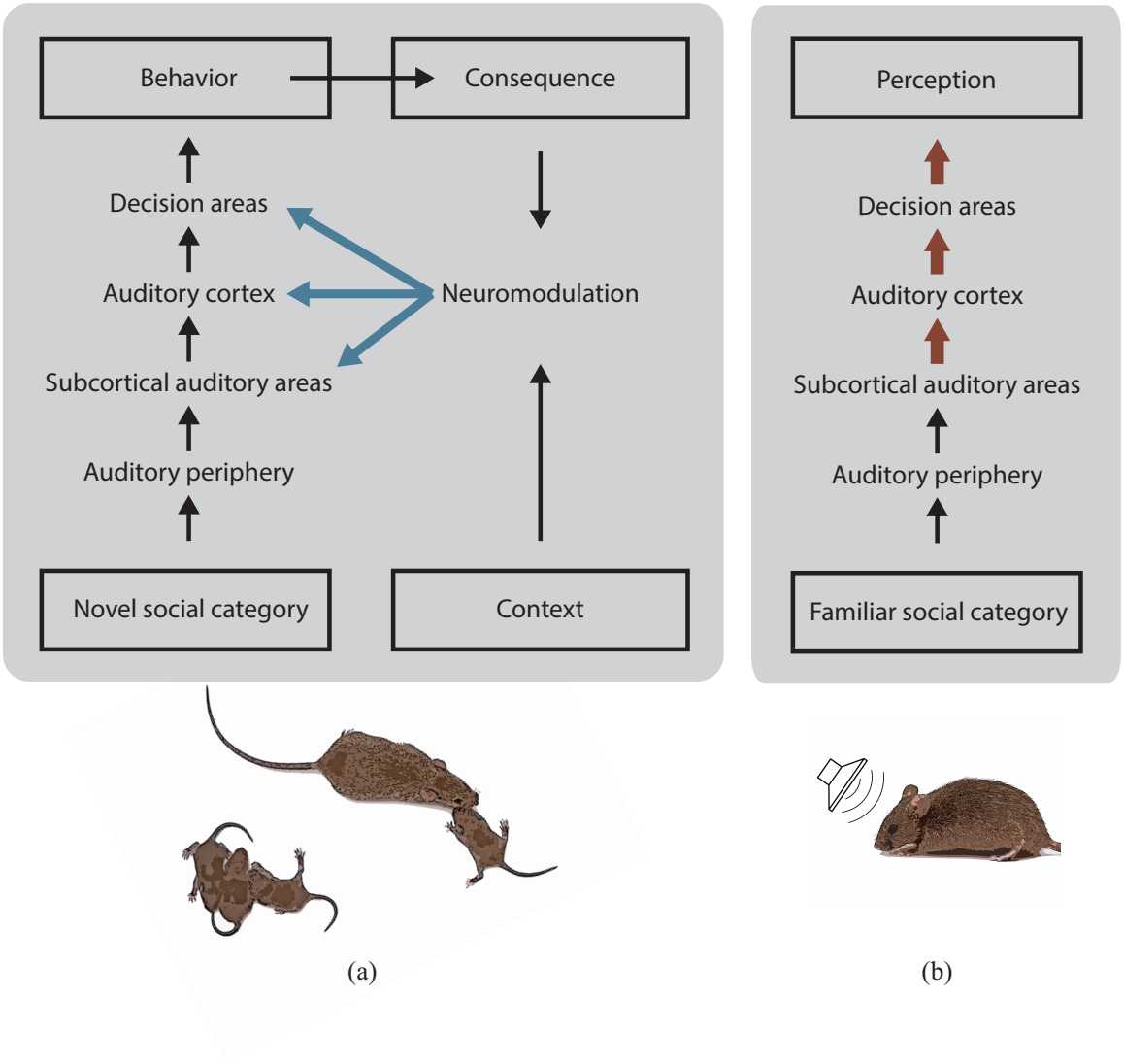


Figure 1.4: A conceptual framework for plasticity in the maternal mouse model. **(a)** During behavior, the release of hormones and neuromodulators driven by reward, contextual cues, and maternal state drive plasticity through various mechanisms such as disinhibition and NMDA receptor mediated long term potentiation. This results in dynamic forms of plasticity that occur at the time of the social interaction. **(b)** The consequence of this model is hypothesized to be an altered feed-forward processing stream in the auditory system that allows awake and passively listening mice to better recognize social sound categories. Adapted from Fig. 3 of Dunlap and Liu (2018).

of pyramidal neurons in core auditory cortex through disinhibition by reducing the spontaneous and evoked activity of genetically-tagged parvalbumin interneurons in lactating mothers (L. Cohen and Mizrahi, 2015). Pup-odor induced modulation does not occur for completely naive virgins, demonstrating a mechanism of plasticity that depends on experience and potentially maternal state. This pup-odor induced disinhibition is markedly different from other multimodal modulation of auditory cortex (e.g. vision or somatosensation) in that it occurs slowly, suggesting that it may not reflect direct projections from olfactory centers into A1, but perhaps operates through indirect neuromodulatory pathways. Hence, it may be that the olfactory system engages neuromodulation to "teach" the auditory system about the behavioral relevance of social sounds.

One possible neuromodulator that pup odor may engage is oxytocin (Munetomo, Ishii, Miyamoto, Sakuma, and Kondo, 2016; Nagasawa, Okabe, Mogi, and Kikusui, 2012), whose release has been shown to play a key role in driving auditory cortical plasticity in response to pup calls. Using the maternal mouse model Marlin and colleagues revealed that disinhibition can be activated with the release of oxytocin into auditory cortex by temporarily weakening the amplitudes of evoked inhibitory postsynaptic currents (IPSCs). This disinhibition results in a balancing of excitatory and inhibitory input of auditory cortical neurons, leading to stronger and more precise responses to social auditory cues through NMDA receptor mediated long term potentiation (Marlin et al., 2015; Mitre et al., 2016). This mechanism might also account for an observed increase in noise correlations between neurons in lactating mothers, which could reflect enhanced synaptic connectivity (Rothschild, Cohen, Mizrahi, and Nelken, 2013).

These multisensory and neuromodulatory influences on auditory cortical responses to pup USVs are likely engaged in both mothers and co-caring virgin mice who are learning to care for pups through experience (Günter Ehret and Koch, 1989; F G Lin

et al., 2013). In mothers, but not cocarers though, the experience translates into more stable long-term changes for the recognition and coding of USVs post-weaning, even in awake mothers outside the pup-rearing context that just passively listen to presented calls (F G Lin et al., 2013). What mechanisms might help stabilize long-term auditory cortical changes initiated by pup interaction? Interestingly, recordings in maternal mice find that the spontaneous activity of a physiologically distinct subgroup of USV-responsive pyramidal neurons is decreased with respect to non-maternal mice (F G Lin et al., 2013; Shepard, Lin, et al., 2015). Such reduced spontaneous activity could diminish the likelihood that spike timing dependent plasticity during other experiences would degrade the USV representation. Additionally, the maternal state induces high levels of estrogen which act to prime auditory cortex to form a more stable encoding of sensory cues (Banerjee and Liu, 2013). Indeed, estrogen has been shown to enhance the recognition of pup calls (Günter Ehret and Koch, 1989).

Overall then, the maternal mouse model has proven a useful paradigm for investigating dynamic as well as long-term mechanisms of auditory cortical plasticity for social stimuli, though many open questions remain. First, while oxytocin release into left auditory cortex is sufficient to accelerate the recognition of and plasticity for pup isolation USVs in naive mice, whether it is necessary in mothers is not known. Additional redundant mechanisms may act endogenously with oxytocin release to facilitate the recognition of pup calls. Second, discrepancies in the literature in how plasticity for USVs manifests across the auditory cortical microcircuit remain to be reconciled. For example, some have found reductions in spontaneous firing for neurons responding to calls in maternal mice (F G Lin et al., 2013; Shepard, Lin, et al., 2015), while others have reported increases (L. Cohen and Mizrahi, 2015), though this could be due to the time point (lactating versus post-weaning) or the cortical layer of the cells. Finally, knowledge about USV responses in higher-order auditory cortical fields is limited, but necessary to understand how neural representations of

variable USVs go on to drive consistent behavioral responses.

1.7 Is maternal auditory cortical plasticity shaped by associative learning?

The maternal mouse model has been successfully used to identify plasticity within auditory cortex for sounds that acquire behavioral relevance, such as pup vocalizations, through social interaction. However, alternative hypotheses could be proposed where the observed changes are dependent on mechanisms that do not require associatively learning the meaning of vocalizations. Such mechanisms are already thought to act in subcortical circuits in the maternal context. For example, with optogenetic activation of a subset of neurons within the MPOA of virgin animals, it is possible to alter neuronal processing to spontaneously drive parental behavior in mice that have not had adult experience with pups (Z. Wu, Autry, Bergan, Watabe-Uchida, and Dulac, 2014). One way for how these neurons might become activated in the context of natural maternal behavior, is that the physiological state of being a mother, due to hormones for example, endogenously changes how active these maternal motivation circuits are, so that the system is primed to respond to infant cues. Research on maternal behavior across species has demonstrated that maternal hormones, such as estrogen and oxytocin, can effect subcortical circuits to facilitate maternal responsiveness (F. A. Champagne et al., 2003; C A Pedersen et al., 1994). These maternal hormones could also be acting similarly within auditory cortex to drive plasticity for pup USVs without explicitly being shaped by their acoustics. This highlights a key difficulty with the maternal mouse model, that sound associations are only being formed for pup USVs, a sound for which its acoustic properties cannot be systematically varied. One would need a paradigm to pair pup interaction with a novel sound that is under experimenter control to test if auditory cortical plasticity is shaped by the acoustics of this novel associated sound. Such a paradigm would allow the full uti-

lization of the maternal mouse model as a platform to uncover biological mechanisms for plasticity to new social sounds.

1.8 Summary and objectives

The maternal mouse model has emerged as a tool for investigating auditory cortical plasticity for sound associations formed during social interactions. This is of interest to auditory neuroscientists, who seek to study auditory cortical processing of sounds in an ethological context. However, in order to further understand the mechanisms of auditory cortical plasticity during social interactions, as well as the role of this plasticity in driving social behavior, we now need the ability to manipulate the acoustic properties of the associated sound. This is not possible with the maternal mouse model since the auditory association is formed with pup ultrasonic vocalizations. This motivates a new behavioral paradigm for pairing a novel sound with pups where the acoustic properties of this sound are under experimenter control. Additionally, this method of pairing needs to be done in such a way so as to allow for the characterization of auditory cortical activity. The central aim of this thesis is the development of a behavioral paradigm that satisfies these objectives.

we first determine whether virgin mice will conesutivly retriev pups as a ... to condition them to relilably approach an auditory In chapter 2, we first characterize how many pups virgin mice will consecutively retrieve as a possible means for conditioning them to approach, with a stereotyped behavior, an auditory stimulus for pup reward. We then developed a pairing paradigm where we tested if the mice could learn to use a novel sound to guide their approach to a target arm at the end of a T-maze for pup reward. In chapter 3, we investigated whether auditory cortical activity was being used to shape this approach behavior by bilaterally silencing it with muscimol in trained mice. in ch 3 we investigated whether approach behavior required audiory cortical activity by In chapter 4, we investigated whether motherhood

impacts learning and memory when mice are trained on our paradigm by comparing performance and approach latency for mother and cocaring virgin mice. In chapter 5, we summarize the results of this dissertation and discuss future questions for which our paradigm can answer.

CHAPTER 2
PARADIGM TO ASSOCIATE NOVEL SOUND WITH SOCIAL REWARD

2.1 Introduction

The onset of maternal behavior in mice has been shown to co-occur with the onset of a preferential approach behavior towards pup ultrasonic vocalizations. By interacting with pups in their home cage, female mice "learn" to preferentially approach distant speakers playing back ultrasonic models of pup calls over playback of other sounds with different acoustic properties, presumably because they recognize the calls as behaviorally relevant. This preference does not occur in naive virgin mice that have not had pup experience, and takes several days to emerge in such animals, while the process is accelerated in mothers (Günter Ehret, 2005). This onset also occurs during a period when extensive plasticity is taking place within auditory cortex (Marlin et al., 2015; Shepard, Lin, et al., 2015; L. Cohen et al., 2011). This plasticity is presumably the result of associative learning mechanisms altering the cortical representation of auditory pup cues to support recognition. However, the neural mechanisms within auditory cortex that support the learning of social communication sounds are not fully understood. One particular point of uncertainty is whether the preferred approach to pup USVs is caused by experience dependent learning or if it is the result of the releasing of a hardwired behavioral program. It has been demonstrated that the activation of small groups of neurons can control innate social behaviors such as aggression and mating (Anderson, 2016). Such circuit nodes are shaped by evolution rather than by experience dependent plasticity. In the context of parental behavior, a subset of galanin-expressing neurons withing the medial pre optic area (MPOA) of the

hypothalamus, when activated, drive pro-parental behavior (Z. Wu et al., 2014). The result from Wu and colleagues suggests that even in non-parental mice, the circuitry for parental behavior already exists, and it just needs to be activated to "unlock" the behavior.

There are several questions the sensory neuroscience community would like to explore regarding these kinds of behaviors. First, we want to study what degree of flexibility exists in these parental behaviors once they have been unlocked. We would like to ask if a novel sensory stimulus can come to drive the subset of galanin-expressing neurons that control the behavior, or if it can affect the behavior by influencing neurons downstream from this population. We would like to ask whether this behavior is constrained to be drivable only by stimuli with certain ethological properties. Specifically for the auditory neuroscience community we would like to study if auditory cortical activity influences these parental behaviors and how auditory cortical representations change in order to allow for new associations to be formed during parental interactions. We would like to study what mechanisms govern auditory cortical plasticity during parental interactions and how motherhood can enhance these mechanism.

One bottleneck in investigating these questions is the lack of appropriate behavioral paradigms. In particular, we need to be able to characterize auditory cortical activity during parental interactions, at the time when auditory associations are being formed. In order to characterize auditory cortical activity we need to present sound stimuli in a controlled and reproducible manner over hundreds of trials. This presents a challenge since social interactions between adult mice and pups typically progress in a manner that does not easily allow for many repetitions of controlled sound delivery. Hence, pairing novel sound with pups, subject to the constraint that the mice produce a stereotyped behavior over enough trials to characterize auditory neurons within an hour, can be challenging. As we explain in this chapter, one possible solution to

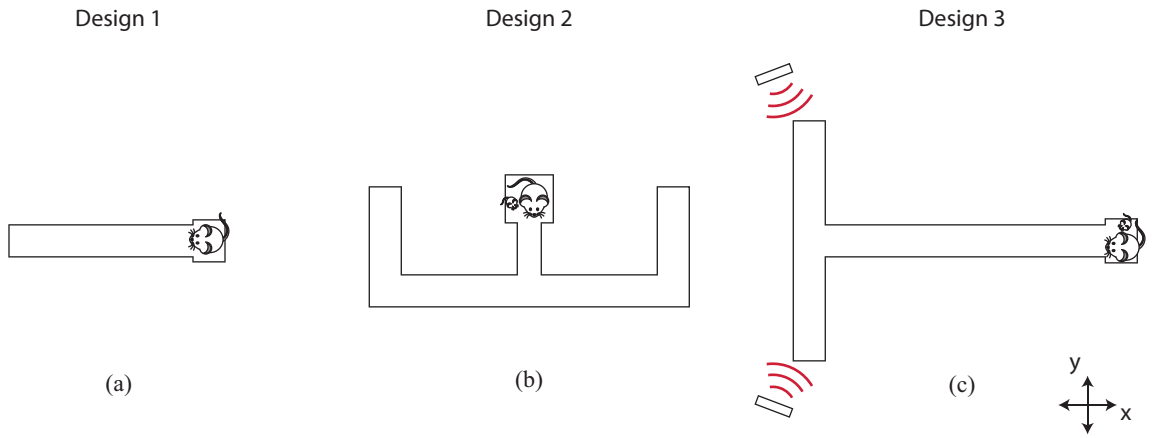


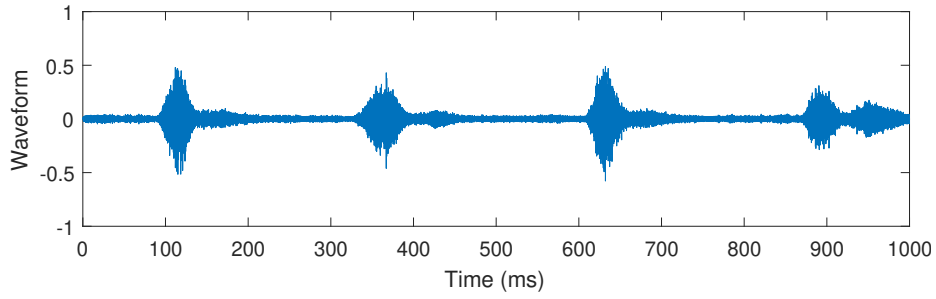
Figure 2.1: The three different maze designs used in this chapter. **(a)** A linear maze was used to quantify the number of continuous retrievals mice would perform. **(b)** A W-maze was used to condition mice to approach a multi-modal stimulus. **(c)** A T-maze was used to condition mice to approach an auditory stimulus.

this problem is to exploit the social interaction of retrieval between female mice and mouse pups. This chapter presents the results of three experimental paradigms using three different maze designs to refine and validate a behavioral paradigm to study the roles of auditory cortical activity and motherhood in learning a novel social sound.

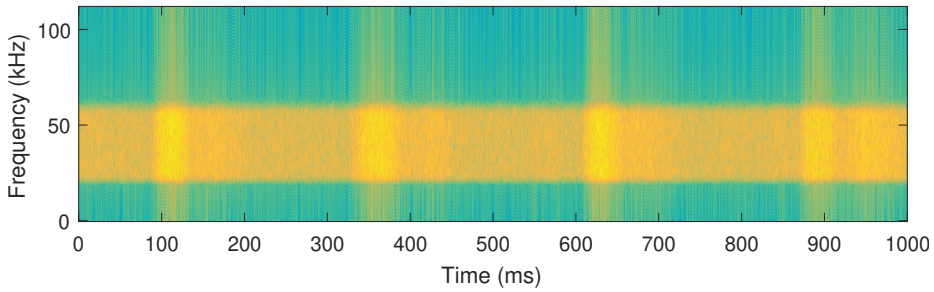
2.2 Methods

2.2.1 Maze designs

All behavioral studies were conducted inside an 80'-2" × 10'-6" double wall anechoic chamber (IAC, Bronx, NY) under dim red light. Animals were tested on one of three different elevated maze designs (refer to Fig. 2.1 for a schematic of the mazes). Design 1 (Fig. 2.1a) was a linear maze with the following dimensions: 30 cm from ground, 54 cm overall length, 2.5-cm-high walls, 46 cm arm length, 8 cm arm width, 8 cm nest length, 10.5 cm nest width, 1 cm drop from arm floor to nest floor, and 50 cm between end of maze and center of nest. Design 2 (Fig. 2.1b) was a W-maze with the following dimensions: 30 cm from ground, 80 cm overall length, 3.5-cm-high walls, 33 cm between the long edge at the base of the W to nest in the y-direction (e.g.



(a)



(b)

Figure 2.2: The auditory stimulus mice were conditioned to approach for pup reward. **(a)** One second sample of the stimulus waveform. **(b)** One second sample of the stimulus spectrogram.

perpendicular to the base of the W), and 30 cm from long edge to the end of the open arms. Design 3 (Fig. 2.1c) was a T-maze with the following dimensions: 30 cm from ground, 86 cm overall length from base to top of the T-maze, 2-cm-high walls, 8 cm arm width, 11 cm nest width, and 1 cm drop from arm floor to nest floor. For design 3, speakers were located 25 cm along the y-direction from the end of the arms at the top of the T-maze and 10 cm along the x-direction from the end of the top of the T-maze. Speakers were oriented so that they faced the decision point at the center of the top of the T-maze. Prior to each test day, mazes were wiped down with Virkon disinfectant and clean Alpha-Dri bedding was added.

2.2.2 Stimulus generation

In order to generate a salient multi-modal stimulus, which was used in paradigm 2, the experimenter would tap with one finger on the Alpha-Dri bedding at one of the ends

of the maze arm (Fig. 2.4). In paradigm 3, amplitude modulated band-limited noise was used as a purely auditory stimulus. This stimulus was designed to be a model of our hand tapping stimulus (same amplitude modulation) but with a center frequency and bandwidth under parametric control. A 2 minute recording of hand tapping was taken with a B&K (Bruel and Kjaer, Naerum, Denmark) microphone (model 4939) connected to a (B&K 2669) preamplifier and (B&K 2690) conditioning amplifier (gain 1V/Pa; filter 20 Hz to 100 kHz) and transmitted to a TDT (Tucker Davis Technologies, Gainsville, FL) RX6 multi-function processor where it was sampled at 223,210 Hz. Custom code designed using TDT's OpenEx software suite interfaces a PC computer with the RX6 to stream the sounds to a data tank. Custom MATLAB (Mathworks, Natick, MA) scripts import the recorded audio from the tank and extract its instantaneous envelope using the Hilbert transform. A vector of Gaussian white noise with equal length to the audio recording was bandpass filtered (center frequency = 40 kHz, bandwidth = 33 kHz) and multiplied element-wise in time by the extracted amplitude envelope to create the final signal. From this final signal, two 20 second subsets were extracted to create "sound examples 1" and "2." During playback the 20 second stimuli were streamed in a constant loop. Example 1 was used for training on days 1 - 7, and example 2 was used on day 8 for testing. For a given trial (see paradigm 2 below), the intensity of the sound at the decision point of the T-maze was fixed, while across trials it was roved between 17 and 30 dB SPL.

The sound stimulus was delivered by a TDT System 3 RX5-2 module, allowing the user to control trial initiation in real time via a custom made remote located inside the training room. The sound was played from Pioneer speakers (model PT-R4), elevated 5 cm above the T-maze, oriented down and towards the decision point (see design 3 in Fig. 2.1c).

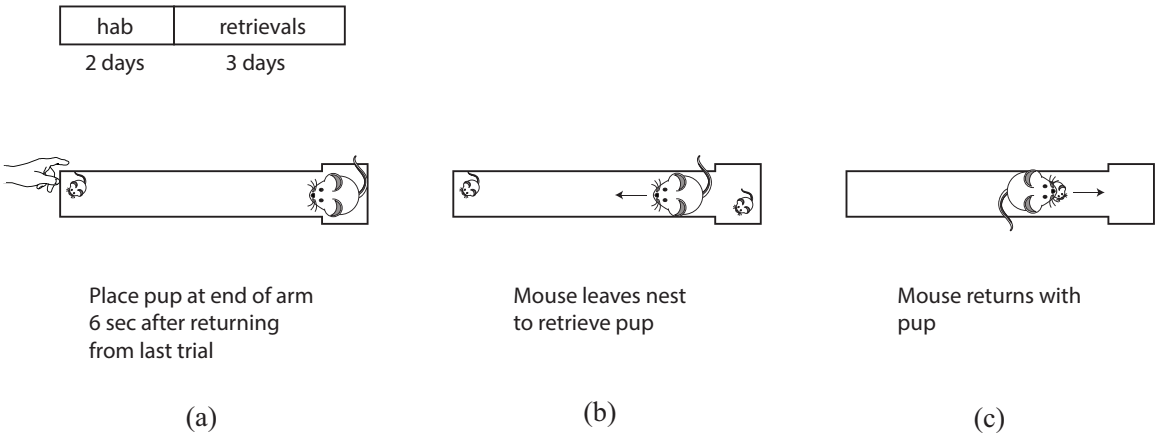


Figure 2.3: Schematic of paradigm 1. **(a)** Six seconds after the mouse has retrieved a pup back to the nest, another one is placed at the end of the linear maze arm. **(b)** The time when the mouse leaves the nest to retrieve the pup is recorded. **(c)** The time when the mouse returns to the nest is recorded. This is also when the 6 second timer is started for the next trial.

2.2.3 Training paradigms

Paradigm 1

We designed paradigm 1 to quantify how many pups a mouse would retrieve if they were continually delivered at the end of a linear maze and to characterize the mouse's approach behavior. CBA/CaJ virgin mice, aged 10-16 weeks, began 2 days of habituation on our linear maze. Habituation consisted of 10 minute sessions during which the experimenter would stay inside the anechoic chamber, occasionally opening and closing the chamber door every 2 minutes to acclimate the mouse to the experimenter's presence and fluctuations in background dB SPL from entering and leaving the testing chamber. Retrievals began after habituation on the third day and lasted for an additional 3 days. During these days, the mouse was initially placed on the maze with two pups present for 10 minutes. If during these 10 minutes a mouse attacked the pups, then that subject was removed from the study. If it did not attack the pups, then we began 40 minutes of continuous retrievals. To initiate the retrievals, one pup was removed from the nest and placed at the end of the linear maze. Once

the mouse left the nest to retrieve this pup, the pup that remained in the nest was removed by the experimenter. Upon returning to the nest with the retrieved pup, the experimenter started a 6 second timer, and when this time was up they placed the pup that they had just removed from the nest at the end of the linear maze. This resulted in a continuous cycle of pup retrievals for the entire 40 minute duration.

Video of the behavior was recorded overhead using a Panasonic color cctv camera (model WV-CP284) mounded to the ceiling and connected to a PC located outside the anechoic chamber, which was running TopScan video tracking software (Cleversys, Reston, VA). TopScan was used to identify the frames where the mouse crossed a threshold located halfway (23 cm) down the linear maze arm. Custom MATLAB code was used to identify the head location and orientation for these frames. Additionally, the time points when the mouse left from and returned to the nest were manually identified from the videos. This data was collected into a table, where for every retrieval we had a row with head position and orientation information as well as key time points of the behavior. At this point, preprocessing was complete and summary statistics were computed using the R programming language (R Core Team, 2017).

Paradigm 2

We designed paradigm 2 for mice to learn to associate a new, multimodal stimulus to guide them to pups. CBA/CaJ virgin mice, aged 10-16 weeks, began 2 days of habituation on our W-maze. Habituation consisted of 10 minute sessions during which the experimenter would stay inside the anechoic chamber, occasionally opening and closing the chamber door every 2 minutes to acclimate the mouse to the experimenter's presence and fluctuations in background dB SPL from entering and leaving the testing chamber. Conditioning began after habituation on the third day and lasted for an additional 3 days. During these days, a mice was initially placed on the maze with two pups present for 10 minutes. If during these 10 minutes the mouse attacked

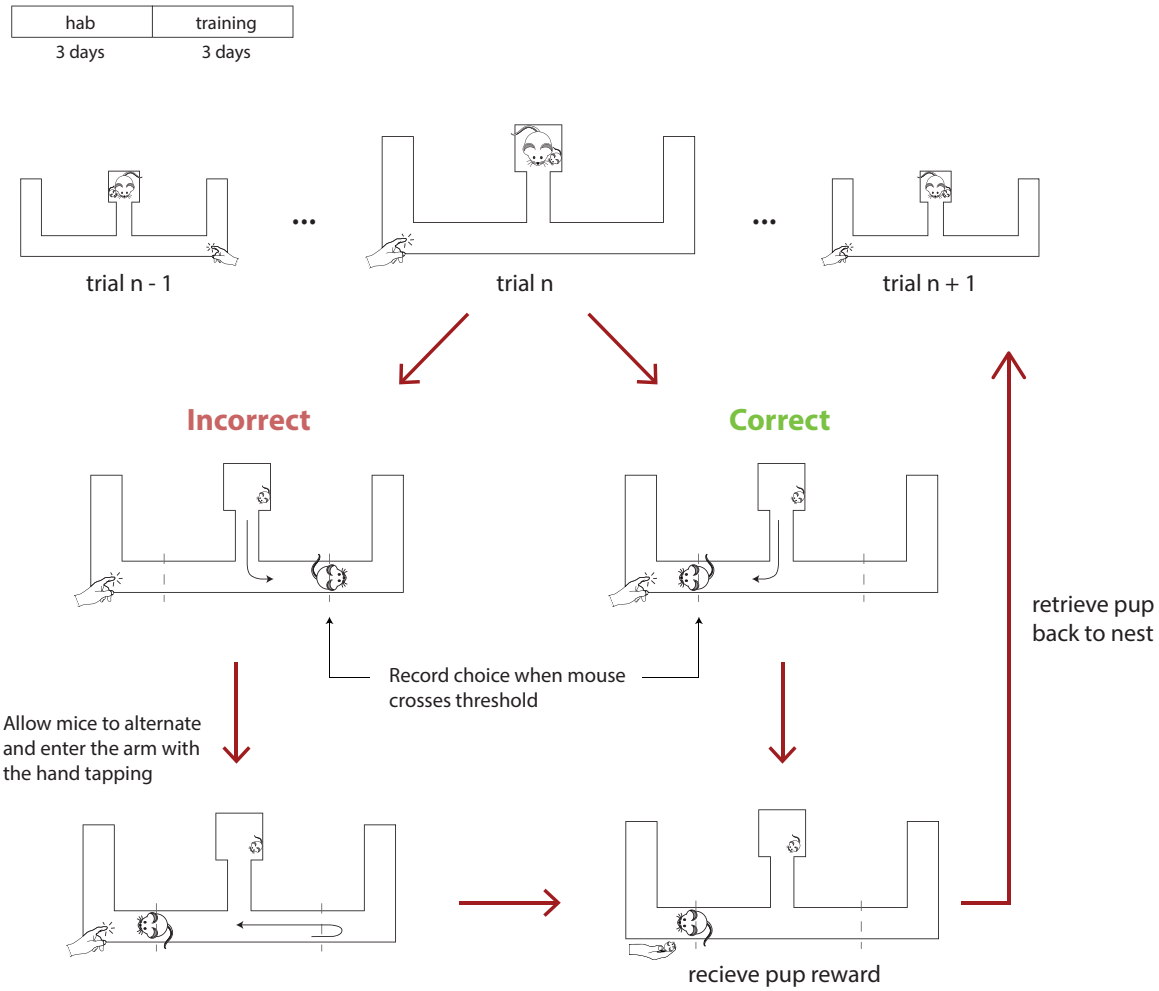


Figure 2.4: Schematic of paradigm 2. Mice are conditioned to approach a multi-modal stimulus (hand tapping on Alpha-Dri bedding) for pup reward.

the pups, that subject was removed from the study. If it did not attack the pups, then we began conditioning. Conditioning consisted of continuously tapping on the side of the W-maze the mouse was supposed to approach (Fig. 2.5). If the mouse correctly decided to approach this side they were rewarded with a pup and the pup which remained in the nest was removed to use for the next trial before the mouse returned with the rewarded pup. If the mouse decided to approach the wrong side the experimenter continued tapping and the mouse was allowed to alternate to the correct side where it would still receive a pup for reward. Conditioning continued for 40 minutes or until the mouse completed 100 trials, which ever occurred first.

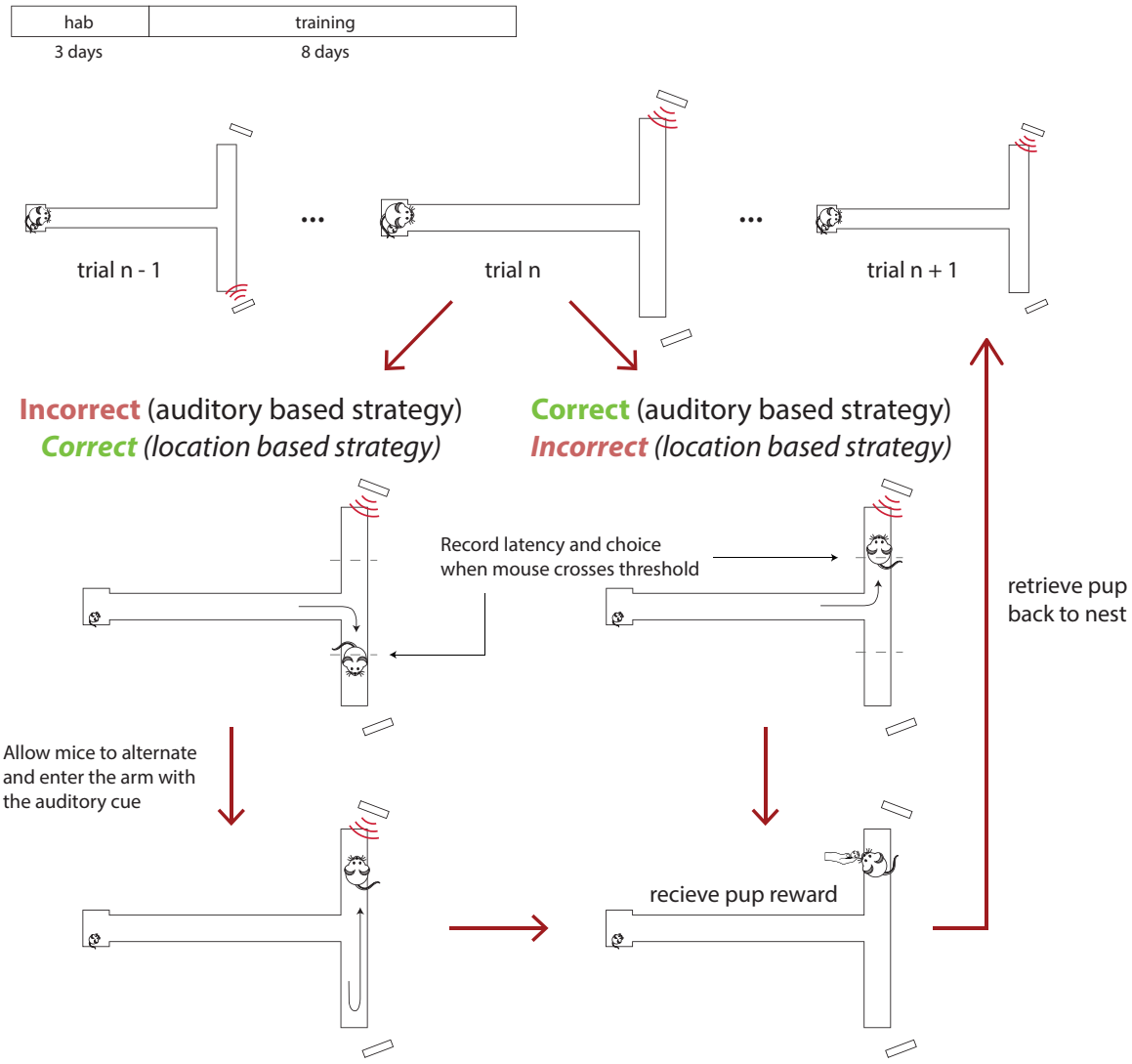


Figure 2.5: Schematic of paradigm 3. Mice are conditioned to approach an auditory stimulus (Fig. 2.2) for pup reward.

Video of the behavior was recorded overhead using a Panasonic color cctv camera (model WV-CP284) mounded to the ceiling and connected to a PC located outside the anechoic chamber, which was running TopScan video tracking software. From the videos each trial was manually scored as correct or incorrect. Summary statistics were computed using the R programming language (R Core Team, 2017).

Paradigm 3

We designed paradigm 3 for mice to learn to associate a new, exogenous sound cue to guide them to pups. CBA/CaJ virgin mice, aged 10-16 weeks, began 3 days of habituation on our W-maze. Habituation consisted of 10 minute sessions during which the experimenter would stay inside the anechoic chamber, occasionally opening and closing the chamber door every 2 minutes to acclimate the mouse to the experimenter's presence and fluctuations in background dB SPL from entering and leaving the testing chamber. Conditioning began after habituation on the third day and lasted for an additional 8 days. During these days, a subject mouse was initially placed on the maze with two pups present for 10 minutes. If during these 10 minutes the mouse attacked the pups, the subject was removed from the study. If it did not attack the pups, then we began conditioning. Each trial started by playing the auditory cue from one of the two speakers when the mouse was in the nest (Fig. 2.5). The speaker side was chosen pseudo randomly with probability 0.5. However, if the same side was chosen consecutively on the three previous trials, then the next side was forced to alternate. A red LED, elevated 30 cm above the maze and blocked from the subject's direct view, would indicate to the experimenter which side the sound was being played from. Once the mouse left the nest and crossed a threshold within one of the two arms, we recorded the latency (measured as the time to cross threshold relative to the time the trial started) and the side that was chosen.

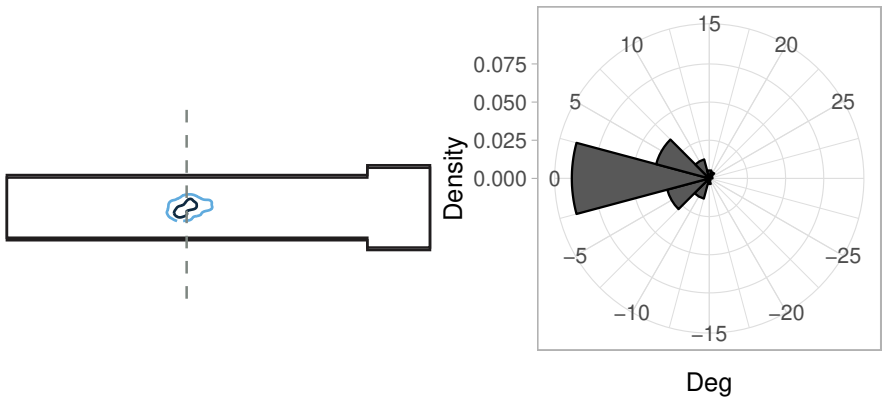
Once the mouse found the correct arm she was rewarded with a pup, which she retrieved back to the nest to initiate the next trial. Before the mouse returned with the retrieved pup, the pup that had been left in the nest was removed and held outside he maze to be used in the next trial. Training lasted for 50 minutes or until the mouse completed 100 trials, whichever occurred first. Trials were scored according to two possible strategies. If the mouse chose the arm that sound was playing from on that trial, the trial was scored as correct according to an "auditory based strategy". If the

mouse chose the arm from which they received a pup on the last trial, the trial was scored as correct according to a "location based strategy".

Mice were trained for 8 days. During the first 7 days, they heard sound example 1. On the 8th day sound example 2 was used. The purpose of the second sound example was to avoid pseudo replication and verify that the mice would learn to generalize to approach sounds with similar acoustic properties and not just some very specific feature of the trained stimulus. Additionally, on the 8th day for 20% of the trials (randomly chosen), no sound was delivered to either speaker in order to verify that performance was based on using the sound. Mice that showed low motivation (stopped retrieving for a whole session) were removed from the study.

Video of the behavior was recorded overhead using a Panasonic color cctv camera (model WV-CP284) mounted to the ceiling and connected to a PC located outside the anechoic chamber, which was running TopScan video tracking software. Custom MATLAB code was used to identify the time points when the mouse crossed the threshold and made a choice. This data was collected into a table where for every retrieval we had a row for latency, the side the sound was playing from, and the side the animal chose. At this point, preprocessing was complete and summary statistics (trial scores) were computed using the R programming language (R Core Team, 2017).

For each trial, the first arm whose threshold was crossed was recorded as a boolean vector CHOICE = left or right. This vector was then converted into a performance vector CORRECT = true or false, depending on the particular strategy, "auditory" or "location" based. Individual mice were classified as having learned the task by pooling together the CORRECT vectors (according to the auditory strategy) from the last two days of training, and testing against the null hypothesis that the components were generated from an independent and identically distributed (i.i.d.) Bernoulli distribution (p=.5) using a one-sample proportion test ($p < 0.5$). A two-sample proportion test was used to test if the performance on sound trials was significantly



(a) (b)

Figure 2.6: **(a)** Schematic of linear maze with 50% (black) and 95% (blue) contour lines for the distribution of x and y head position coordinates at the time the mouse crossed the halfway threshold detected by TopScan. **(b)** Circular histogram of head angle relative to x-axis (n = 750).

different from silent trials.

2.3 Results

2.3.1 CBA/CaJ virgin mice will repeatedly retrieve pups

We used paradigm 1 to quantify how many pups a mouse would retrieve if they were continually delivered at the end of a linear maze and to characterize the mouse's approach behavior. We found that this paradigm produces a steady, stereotyped retrieval of over 100 pups in a 40 min time period with no indication of stopping. Additionally, mice exhibited little variability in their head position during the approach (Fig. 2.6), ensuring that sound can be delivered consistently to within only +/-3 dB SPL for ultrasound frequencies, and less for lower frequencies. Head angle varied within +/-15 degrees from the approach axis, an angular difference that has been shown to be perceptually indistinguishable for mice (Heffner, Koay, and Heffner, 2001). Hence, we would be able to collect an appropriate number of consistent trials to characterize auditory neural activity in a reasonable time frame for holding well

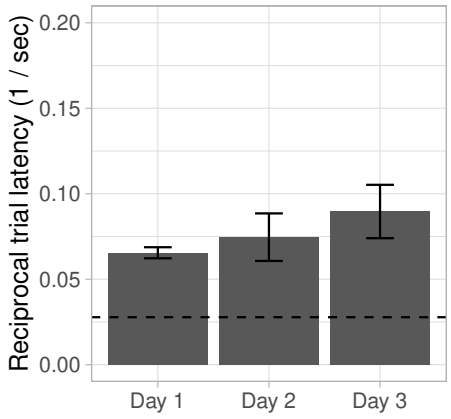
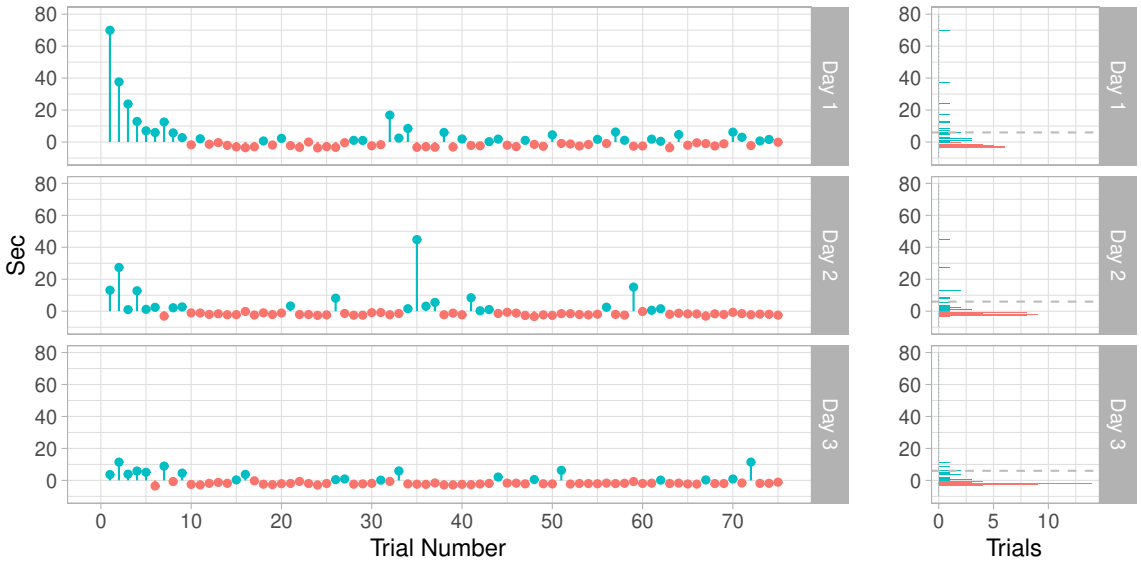


Figure 2.7: Average reciprocal trial latency for group (n = 3 mice). Dashed line indicates minimum reciprocal latency needed to achieve 100 trials in an hour.

isolated neurons. We also found that after the initial few trials, once mice were engaged in persistent retrieval, they could leave the nest before any sensory cue was presented in that trial (Fig. 2.8). This suggests that the decision to leave the nest in this repetitive paradigm was being driven for the most part by the internal state of the mouse, rather than by pup cues.

2.3.2 Pairing pup reward with novel mulit-modal stimuli

We used paradigm 2 to test if a mouse could learn to use a novel multi-modal stimulus to guide their approach when continuously retrieving pups. We found with the W-maze of paradigm 2 (Fig. 2.9) that virgin mice could continue to perform retrieval trials at a high rate even when presented with a choice and the uncertainty of receiving a pup in any particular arm. Mice could complete 100 trials in 40 minutes after just 2 days of training. When a mouse entered the incorrect arm and did not receive a pup, it would spontaneously enter the other (correct) arm in rapid succession. Upon presentation of a pup, the mouse would quickly and reliably retrieve it, just as in paradigm 1. With this paradigm virgin mice could learn to achieve high performance with greater than 90% accuracy in 100 trials by the third training day.



(a) (b)

Figure 2.8: Testing continuous retrievals on linear maze (n = 3). **(a)** Average time leaving nest relative to pup delivery. Blue = left nest after pup delivery. Red = left nest before pup delivery. **(b)** Distribution of latency to leave nest. Dashed lines indicate when pup was placed on the arm.

2.3.3 Pairing pup reward with novel auditory stimuli

We used paradigm 3 to test if a mouse could learn to use a novel auditory stimulus to guide their approach when continuously retrieving pups. With paradigm 3, when we restricted the stimulus to being purely auditory, mice took significantly longer to reach a level of performance above chance (Fig. 2.10). We found that after 7 days of training 9, (69%) of our virgin mice met our criteria for having learned the task, with an average performance on day 7 of 72%. On day 8, when tested with sound example 2, average performance for learners was 76% during playback trials and 47% for silent trials. This confirmed that learners were in fact using the auditory stimulus to guide their choice for which maze arm to enter, and that their association generalized to new sounds with similar acoustic properties. For learners, average reciprocal latency increased over training, unlike for non-learners, putatively unmotivated mice.

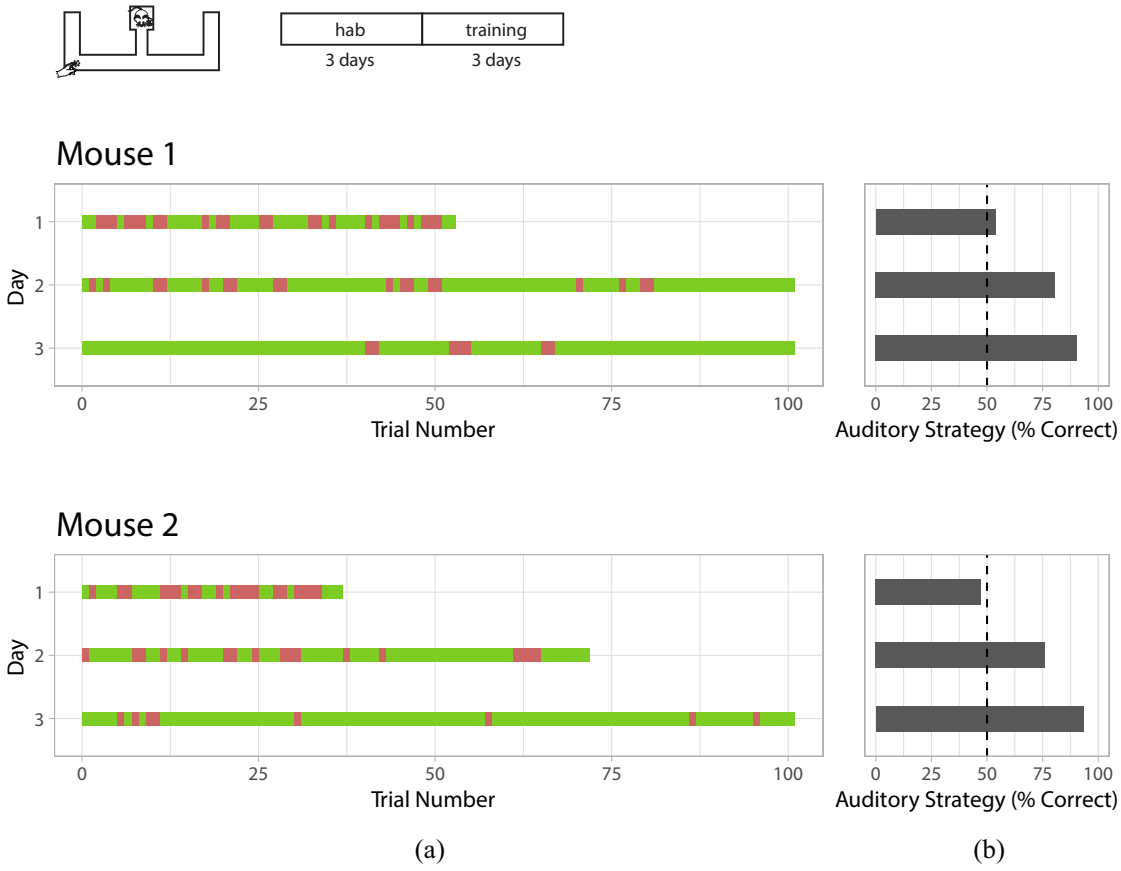


Figure 2.9: **(a)** Performance scores over 40 min daily training sessions for individual conditioned mice. **(b)** Individual daily performance estimates for individual conditioned.

2.3.4 Virgin mice display a shift in strategy over training

We found that for early training days, when auditory strategy performance was at chance, mice were not choosing which arm to enter at random. Instead, they had an initial location-based strategy, wherein they entered the last arm they received a pup, which was also the arm they just returned from on their previous trial (Fig. 2.5). If we measure performance based on this initial location-based strategy for an individual animal, it decreased over training for learners but not for the non-learner mice (Fig. 2.12b). By plotting performance of the auditory- and location-based strategies in a 2-dimensional graph, which we call the strategy space, we see how a mouse's strategy shifts over training from being location-based (upper left quadrant)

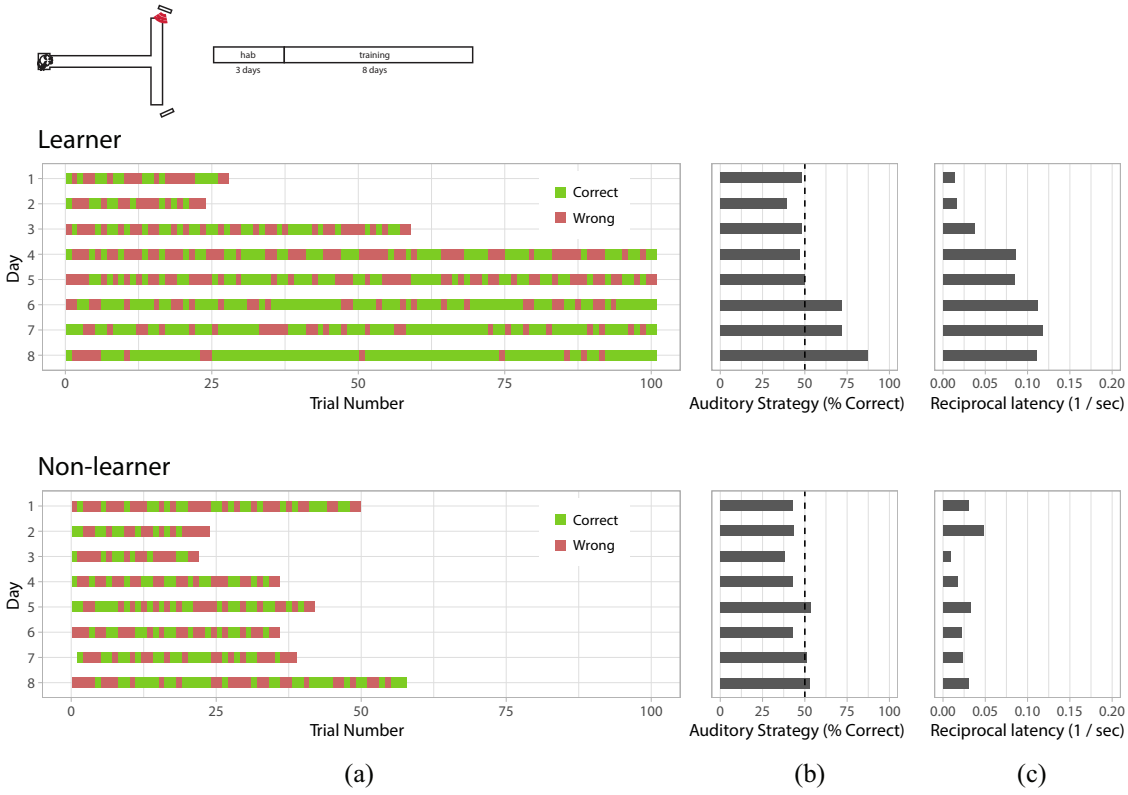


Figure 2.10: **(a)** Performance scores according to the auditory based strategy over 50 min training sessions for exemplars of learner and a non-learner mice. **(b)** Individual performance estimates according to the auditory based strategy for exemplars of learner and non-learner mice. **(c)** Individual reciprocal latency estimates for exemplars of learner and non-learner mice.

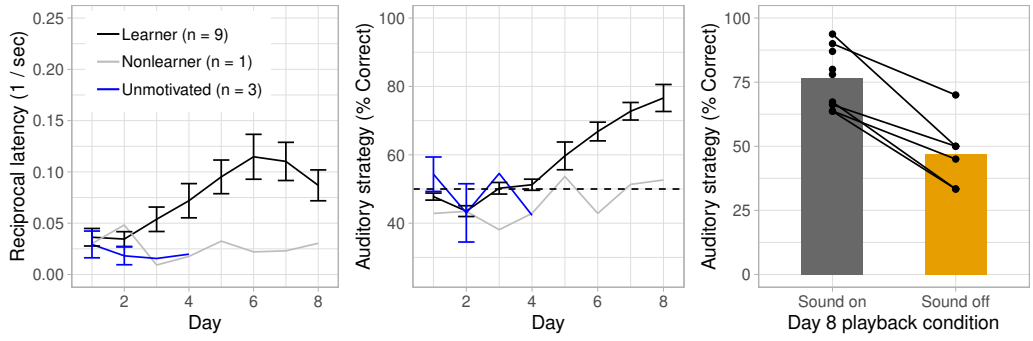


Figure 2.11: **(a)** Group averaged reciprocal latency for all mice. Unmotivated mice were those who were not trained for all 8 days because they stopped retrieving and therefore could not be tested for reaching criteria. **(b)** Group averaged performance for all mice with trials scored according to the auditory based strategy. **(c)** Group performance on day 8 for silent and sound trials scored according to the auditory based strategy (n = 6 Silent, 9 Sound).

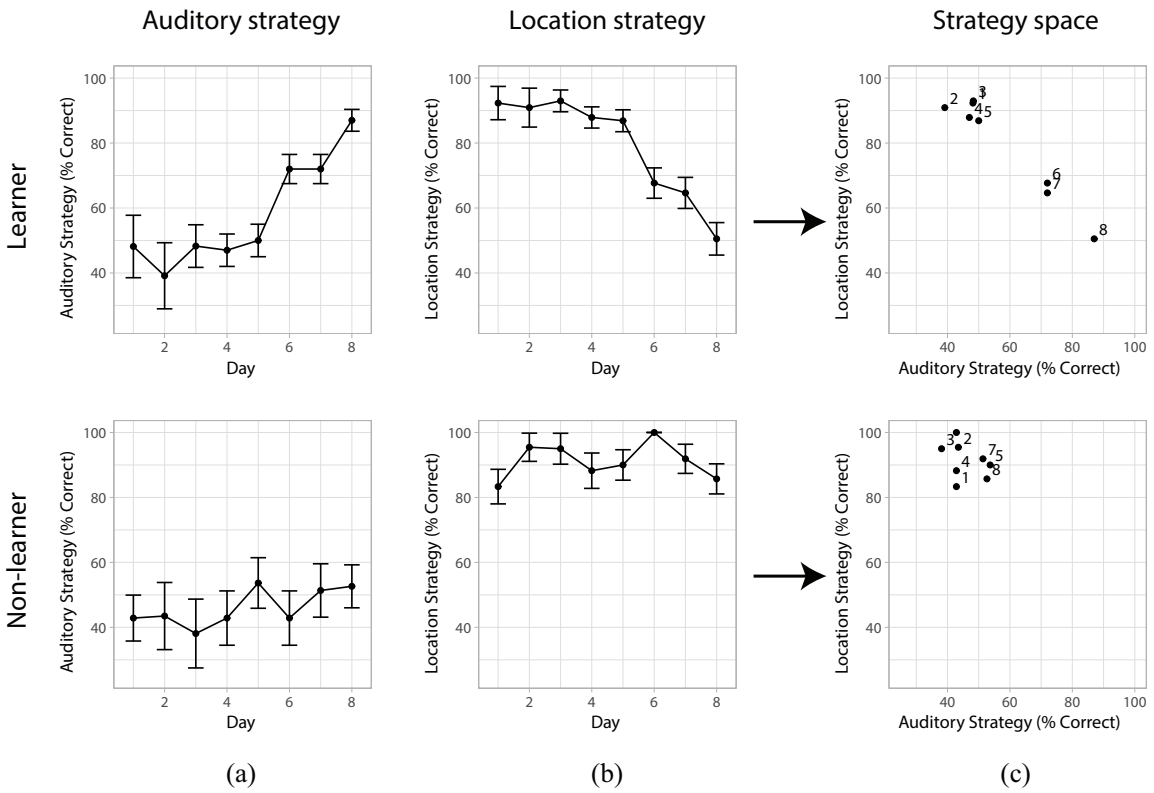


Figure 2.12: Individual performance on learned strategy. **(a)** Individual performance estimates according to the auditory based strategy for exemplars of learner and non-learner mice. **(b)** Individual performance estimates according to the location based strategy for exemplars of learner and non-learner mice. The non-learner mouse continues to choose which arm to enter according to the location based strategy even though it provides no additional advantage in locating the pups. **(c)** Individual performance estimates for both strategies (x-axis = auditory, y-axis = location). For mice that learned the auditory based strategy we see a shift from the upper left quadrant of the graph to the lower right quadrant. Error bars have been removed for clarity.

to being auditory-based (lower right quadrant). Strategy space points for a non-learner remained clustered in the upper left quadrant. The shift from the upper left to the lower right quadrant was still observed in strategy space group averages over learners for each day (Fig. 2.13). Performance for trials where the sound was "off" occupied a different region of the strategy space corresponding to chance for the auditory-based strategy and slightly above chance (60%) for the location-based strategy.

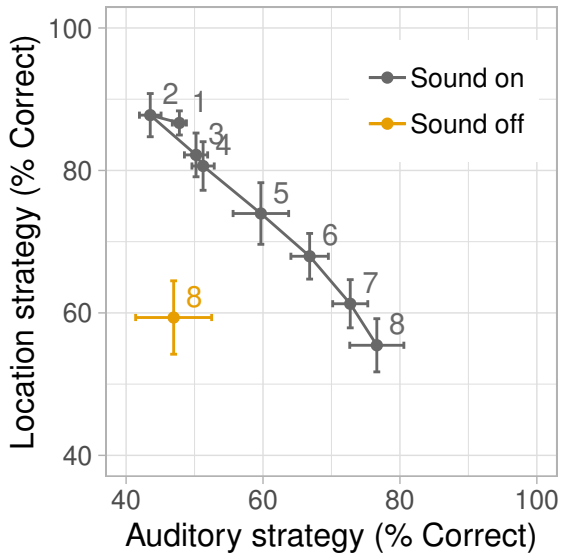


Figure 2.13: Group averaged performance estimates for both strategies (x-axis = auditory, y-axis = location) for learner mice. We see a shift from the upper left quadrant of the graph to the lower right quadrant for trials where sound was playing. We also see a shift for trials were sound was not playing. These trials occupy a different region of the strategy space corresponding to chance for the auditory based strategy and slightly above chance (60%) for the location based strategy.

2.4 Discussion

The auditory neuroscience community has taken an interest in the maternal mouse model because it provides an ethological paradigm for studying social sound learning. Several recent studies have shown that motherhood and pup experience lead to extensive auditory cortical plasticity for pup ultrasonic vocalizations (USVs). Researchers have observed excitatory plasticity in onset and offset responses (Shepard, Lin, et al., 2015), inhibitory responses (Galindo-Leon et al., 2009), connectivity (Rothschild et al., 2013), and multi-sensory processing (L. Cohen et al., 2011). These studies are informative for the kinds of plasticity we expect to occur during learning in our conditioning paradigm. Here we would like to highlight some issues with past studies on either approach responses to pup sounds, or pup-experience induced auditory cortical plasticity that motivated the development of our paradigm.

2.4.1 Motivation for behavioral training paradigm

Early work investigating maternal behavior found that sound processing is not necessary for retrieval in rodents, since deafened rats do not display deficits in retrieving (Beach and Jaynes, 1956). It was later revealed that although acoustic cues from pups were not necessary for retrieval, they were sufficient to initiate retrieval behavior (J. C. Smith, 1976), and that with pup experience, they could be recognized and preferentially approached over non-pup auditory cues (Günter Ehret and Haack, 1982). These results are consistent with our findings. In paradigm 1, we showed that after the first few trials, mice were more likely to preemptively leave their nest to retrieve pups before a pup had been placed on the maze. This demonstrated that sensory cues were not necessary to initiate the search for a pup on a given trial provided the mouse was properly motivated. Although it is known that virgin female mice will act maternal towards pups, the sustained retrieval we observed has only previously been demonstrated in mother ground squirrels (Michener, 1971). We are presenting for the first time sustained retrieval in virgin female CBA/CaJ mice. However, it is quite possible that this behavior differs across strains of mice since unlike rats, maternal behavior in mice is highly strain dependent (Dulac et al., 2014).

Paradigm 3 showed that after conditioning on the T-maze, a novel, non-ethological auditory cue can be sufficient to guide a mouse's approach. However, it is important to note an important difference between our paradigm, and past studies that tested the ability of sound stimuli to initiate approach. Previous work required the mouse to settle in its nest. For example, Ehret's method required that a mouse remain in their nest for 30 seconds before switching on the sound to initiate a trial (Günter Ehret and Haack, 1982). We did not require this, and instead conditioned our mice while they were in a state of constant retrieval.

In our earliest attempts to pair sound with pups (data not shown), we used a similar paradigm where we required our mice to settle back in the nest before initiating

the next trial. We noticed that for initial trials, mice would approach the playback speaker, but they would take a long time (on the order of minutes) to settle back down in the nest after each retrieval. Additionally, after a small number of initial trials (approximately 5), they would quickly habituate to the task, eventually ignoring sound playback altogether and remaining in the nest. This rapid habituation to the task has been previously seen, even for mice that show a preferred approach (Günter Ehret and Haack, 1982). Both of these drawbacks of the earliest studies place limitations on the number of trials that can be completed per mouse per day, thereby posing a major problem for future experiments to characterize neural activity during social approach.

This motivated us to ask how many pups we could get mice to retrieve sequentially, and at what rate would they do this if those pups were continually presented at the end of the maze arm. To address this, we designed paradigm 1 to identify whether mice would preemptively leave the nest during continuous pup retrieval. This approach represented a paradigm shift in how we thought about the role of sound during pup retrieval. It also delineates two distinct types of choices: the choice to leave the nest, and the choices made to navigate to the pup. Importantly, previous paradigms that use approach or pup retrieval as a measure of sound recognition do not make this distinction (Günter Ehret and Haack, 1982, F G Lin et al., 2013, Marlin et al., 2015), highlighting an advantage of our current approach.

Next, in most studies of experience-dependent auditory cortical plasticity for pup USVs, pup-experience is treated as a black box. Because the experimenter does not have control over how the mouse interacts with pups, it is not known what aspects of pup interaction leads to either the formation of preferred approach, or the various forms of plasticity. In our conditioning paradigm, it is possible to condition a mouse that has only experienced pups through our training protocol. This allows future experiments where various brain areas associated with preferred approach could

be optogenetically deactivated at specific times during conditioning. This would allow for the identification of brain areas whose activation is needed to form auditory associations with pups, as well as the specific instances of pup interaction during which these brain areas need to be active. Finally, past studies could only investigate the association formed for sounds that the pup produced. Our paradigm is the first to condition mice to form an association between a pup and a sound that is under the experimenter's control. This has numerous advantages that we discuss below in the section on new questions we can address.

Within the maternal behavior community, it has long been recognized that pups can be used as a potent reward for conditioning rodents. Earlier studies used either pavlovian place preference paradigms (Seip and Morrell, 2007) or operant lever pressing paradigms (Hauser and Gandelman, 1985; A. Lee et al., 2000) to study this. However, none of the prior work attempted to form an auditory association. Our paradigm also has an advantage over conditioning mice to lever press, which requires a shaping period where the rodents learn to associate lever pressing with getting reward. In our paradigm the retrieval behavior the mice are performing is natural, so there is no shaping period required.

2.4.2 A paradigm to investigate the flexibility of an innate behavior

A common dichotomy used to classify animal behavior is the concept of innate versus learned. Innate behaviors are behaviors that don't require experience to be expressed and they also do not change with experience. These behaviors are thought of as being controlled predominantly by genetic factors. On the other hand, learned behaviors are those behaviors that require experience to be expressed and can change with experience. Although behaviors are typically described as being innate or learned, it is appreciated within the neuroscience community that not all behaviors can be so easily distinguished (Grillner and Wallén, 2004). Examples of such behaviors are ones

that are innate in the sense that they don't require experience to be expressed, but are also learned because they can be further refined by experience. Our paradigm falls into this category of being both innate and learned, since the mice don't require experience to use their innate strategy but then shift to a learned strategy with experience. This kind of flexibility in innate behaviors is of interest to the neuroscience community, especially understanding the roles of cortical vs subcortical circuits in this form of learning (Kawai et al., 2015; Grillner and Wallén, 2004; Berkinblit, Feldman, and Fukson, 1986). The paradigm we have developed could be used to elucidate these roles within the context of infant care.

2.4.3 New questions this paradigm can address

Our paradigm enables many new questions to be answered, two of which are the subjects of the following chapters. First, because we can collect hundreds of trials per animal, we now have the ability to identify whether an individual mouse has learned the task and formed an auditory association rather than assess learning only at a group level.. Additionally, because all trials are conducted in a relatively short period (1 hour or less), we can temporarily block activity during this time period in brain areas to identify necessary nodes for social auditory processing. This is done in chapter three, where the reversible GABA agonist muscimol is used to temporarily block neural activity in auditory cortex in mice that had previously learned the task. Since we can now control the amount of pup experience, in chapter four we ask whether motherhood enhances learning while accounting for different levels of motivation across our animal groups.

Beyond what is studied in this thesis, we can use this paradigm to explore numerous additional questions of interest to both the sensory learning and maternal behavior communities. We can apply pharmacological manipulations during learning to test whether various mechanisms of plasticity are necessary for this social auditory

learning. By extending the auditory cortical inactivation study in chapter two to other brain regions, we can deconstruct the neural circuit for social auditory association, similar to what exists for motor learning in the birdsong community. Because the sound stimulus is under experimenter control, we can explore whether mice have a preference for forming social auditory associations with sounds of specific acoustic properties. Presumably such a preference would be the result of hardwired neural circuits favoring ethological stimuli that have been shaped over evolution.

Ultimately we would like to used this paradigm for social auditory conditioning with distractor sounds. This would enable us to conduct preferred approach experiments as a choice between 2 or more sounds, similar to studies done by Günter Ehret (2005). When combined with simultaneous neural recordings, this approach will provide a useful tool for investigating the auditory neural changes that occur online during the learning of social sound categories.

2.4.4 Auditory stimulus deliver from speaker vs hand tapping

Although the reason why mice learn faster with the hand tapping stimulus is not central to the aims of this thesis, the effect is quite glaring, and therefore, worth discussing briefly. A likely reason for faster learning is that hand tapping generated a spectrally more complex sound which was either more salient or easier to localize. There is also the possibility that hand tapping generated non-auditory cues such as vibration or odor which could also be more salient or enhance learning by forming a multi-sensory association. Future studies are required in order to resolve the discrepancy in learning rates for these two training paradigms in this chapter.

CHAPTER 3
THE ROLE OF AUDITORY CORTICAL ACTIVITY

3.1 Introduction

A primary interest of the auditory neuroscience community is to understand how sound is mapped into behavior and how this process can become disrupted by disease. Much progress has been made in understanding how the auditory system transforms sounds into behavior through detecting and forming experience-dependent representations that can support decision making. Hypotheses have been proposed to explain general principles governing the computations to form these representations. For example, the efficient coding hypothesis (Gervain and Geffen, 2018; E. C. Smith and Lewicki, 2006; Lewicki, 2002) predicts how these representations are shaped by the statistics of sounds that the animal has been exposed to. These representations feed into downstream circuits responsible for action selection and decision making, potentially biasing their output. This ability to bias behavior can be both open in that it can be acquired and refined through experience, as well as closed (Mayr, 1974). A central question in auditory neuroscience is to understand the circuitry that controls these processes, auditory plasticity.

A key processing hub in this circuitry is auditory cortex. Studying acoustic representations within auditory cortex specifically has revealed that they are important for a large variety of tasks. These tasks span from sound source localization (Malhotra, Stecker, Middlebrooks, and Lomber, 2008; Malhotra and Lomber, 2007) to representing complex task variables that are more closely related to perception and choice (Niwa, Johnson, O'Connor, and Sutter, 2013; Bizley, Walker, Nodal, King, and Schnupp, 2013; Mamiko Niwa, Johnson, O'Connor, and Sutter, 2012). The discovery

of these more complex representations has led to the concept that auditory cortical representations can be shaped by experience and memory (Christison Lagay, Gifford, and Cohen, 2015; Giordano, McAdams, Zatorre, Kriegeskorte, and Belin, 2013; Bizley and Cohen, 2013).

The paradigm we developed in chapter 2 may form such a representation in auditory cortex for the sound the mice are conditioned to approach. If so, we seek to use this paradigm to further explore these representations within the social context of pup retrieval (Banerjee and Liu, 2013). However, it is possible that the behavioral paradigm we developed in chapter 2 does not engage auditory cortex sufficiently. Although some auditory conditioning paradigms require auditory cortical activity and plasticity (Banerjee et al., 2017), the conditions for which auditory cortical function is needed even in standard auditory conditioning tasks is not well understood (LeDoux, Farb, and Romanski, 1991; Campeau and Davis, 1995; Zhang et al., 2018). One explanation for why the conditions that require auditory cortical processing have been hard to determine, hypothesizes that auditory cortical engagement requires sufficient acoustic complexity (LeDoux, 2000). Since our sound stimulus is simply amplitude modulated broadband noise, it may very well not be "complex" enough to engage auditory cortex. Additionally, the possibility that performance on our paradigm might not require auditory cortical activity is also supported from the anatomy of the auditory system. Auditory thalamus sends direct projections to basolateral amygdala and perirhinal cortex (LeDoux et al., 1991; Campeau and Davis, 1995), brain areas that are part of the pathways for both pup directed aggression and pro-parental behavior (Dulac et al., 2014; Banerjee and Liu, 2013; Simerly and Swanson, 1986). For these reasons, we cannot simply assume that our conditioning paradigm drives a behavior that requires auditory cortical activity.

Although it has not been explicitly tested whether or not auditory cortical activity is needed to respond to auditory pup-cues, various studies suggest that this is likely

to be the case. Early studies by Ehret and colleagues showed that experience caring for pups will lead to a preferential approach to pup-associated auditory cues (Günter Ehret, 2005), one of which also reported enhanced cFos expression in auditory cortex (Fichtel and Ehret, 1999). However, no lesioning of auditory cortex was performed to test if auditory cortical activity was needed for preferred approach. Still, evidence suggests that maternal experience drives plasticity in auditory cortex. Recordings in auditory cortex have demonstrated that this plasticity alters the representation of auditory pup-cues to make them more salient by inhibiting neurons that are tuned to different frequencies and better separate the representation of pup and adult calls. Presumably this plasticity is happening to benefit downstream brain areas within the maternal circuit, suggesting that they do use information that has been processed by auditory cortex.

Further evidence that the auditory cortical representation is needed to maternally respond to auditory pup cues comes from studies performed by Marlin et al. (2015), where it was shown that silencing left auditory cortex blocked pup retrieval. Additionally, it was shown that by activating sound driven plasticity in auditory cortex with the release of oxytocin, non-retrieving mice would spontaneously retrieve. Although this study did not explicitly test for sound recognition, which requires the mice to perform an auditory-based task, it does suggest a role for auditory cortical activity in affecting the parental motivation of mice to retrieve pups. In the previous chapter we saw that motivation to retrieve pups was positively correlated with performance on our auditory task. This suggests that silencing auditory cortical activity would reduce both motivation and performance in our paradigm. We explicitly test this prediction with the experiment conducted in this chapter.

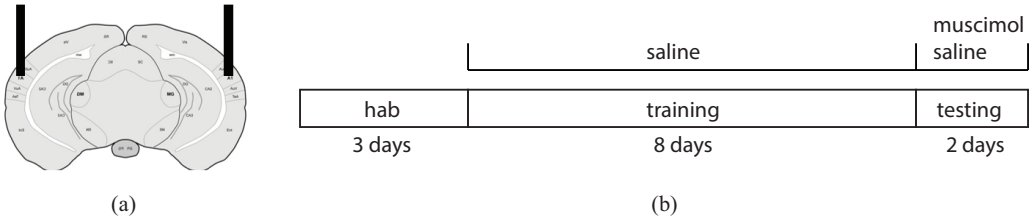


Figure 3.1: Muscimol infusion into auditory cortex experimental design. **(a)** Auditory cortex was bilaterally cannulated (bregma = -3.04 mm, lambda = +/-3.75 mm, z = -0.5 mm from cortical surface). **(b)** Timeline of experiment. On training days, saline was infused 24 minutes prior to conditioning. On testing days, either saline or muscimol was infused 24 minutes prior to conditioning. The experimenter was blind to what treatment was being given.

3.2 Methods

3.2.1 Cannulation of auditory cortex

At least 1 week before the first day of habituation, mice were bilaterally cannulated into auditory cortex (bregma = -3.04 mm, lambda = +/-3.75 mm, z = -0.5 mm from cortical surface). Cannulas were purchased from Plastics One (C315GS-4/SPC guide-cannula, stainless-steel, 26 gauge, 4mm pedestal size, cut length 4mm below pedestal). Mice were implanted with the cannulas under Isofluorane (2-5% with O_2), after they showed no reflexive response to a light toe pinch. Cannulas were implanted to a depth of 500 microns below the cortical surface and covered with a dummy cap (Plastics One, C315DCS-4/SPC). Mice were group housed up until the cannulation surgery, after which they were housed individually as they recovered. During recovery, in order to prevent damage to the implanted cannula, food and water were only delivered through side Bio-Serv liquid diet feeding tubes (model 9019).

3.2.2 Drug infusion

Muscimol was purchased from Sigma-Aldrich in powder form (catalog number M1523). For infusions, solutions of 3.5 mM in saline were prepared from which 0.4 ul (160 ng) was injected over 2 minutes using a World Precision Instruments syringe pump

(model SP100i) with a 25 ul Hamilton syringe (model 702) while the mice were briefly anesthetized with Isofluorane (2-5% with O_2). The drug was allowed to diffuse for 5 minutes after infusion before removing the cannula injector. Bilateral injections were performed serially requiring the mouse to be under Isofluorane for a total of 14 minutes.

3.2.3 Experimental design

All behavioral studies were conducted inside an 80'-2" × 10'-6" double wall anechoic chamber (IAC, Bronx, NY) under dim red light. Seventeen CBA/CaJ mice between the ages of 12 and 16 weeks, and one week after cannula implantation, began 3 days of habituation on our T-maze. Habituation consisted of 15 minute sessions during which the experimenter would stay inside the anechoic chamber, occasionally opening and closing the chamber door every 5 minutes to acclimate the mouse to the experimenters presence and fluctuations in background dB SPL from entering and leaving the testing chamber. After habituation, the mice began training using conditioning paradigm 3 and the sound stimulus previously described in chapter 2 with the difference that before each training session, 0.4 ul of saline was injected bilaterally over 14 minutes according to the infusion protocol described above. After infusion, the mice were given 10 minutes to awake from anesthesia in their home-cage, after which they were placed on the T-maze to begin training paradigm 3 from chapter 2. Mice were classified as having learned the task if their performance pooled across the last two consecutive training days was significantly greater than chance. For the mice that met this criteria, two testing days were performed where the mice received either saline or muscimol before the conditioning session, for which the experimenter was blind.

All mice were virgin with no pup experience prior to habituation. On the last day of habituation, pups were placed inside their homecage for 30 minutes in an effort to

increase the chances that they would be motivated to retrieve on the first conditioning day.

Reciprocal latency and performance for saline and muscimol treated animals were compared using a Generalized Linear Mixed Model (Nelder and Baker, 2004; Wood, 2006) to account for multiple observation per mouse. Therefore, observations for both metrics were modeled as being distributed according to an exponential family. The reciprocal latency of each trial was modeled as being gamma distributed, since it is non-negative. To model performance (auditory- or location-based), the score of each trial was modeled as being binomial distributed.

To test for a difference in reciprocal latency as a function of treatment, we constructed models with 2 fixed effects: an intercept term and a binary treatment term. Each of these terms were also treated as random effects grouped by each animal. To test for a difference in performance as a function of treatment, we constructed models with 2 fixed effects: an intercept term and a binary treatment term. Each of these terms were also treated as random effects grouped by each animal id. These models were fit using the lme4 package (Bates, Mächler, Bolker, and Walker, 2015) in R (R Core Team, 2017).

3.3 Results

Of the 17 mice we cannulated and trained for 8 days, a total of 9 (53 %) met our criteria for having learned the task. These 9 were then tested with bilateral muscimol injections.

3.3.1 The effect of auditory cortical activity on latency

Average reciprocal latency as a function of treatment is presented in figure 3.2 for mice that learned the task. The average latency difference per animal failed a normality test (Shapiro-Wilk $p < .05$) indicating that a paired t-test would not be appropriate.

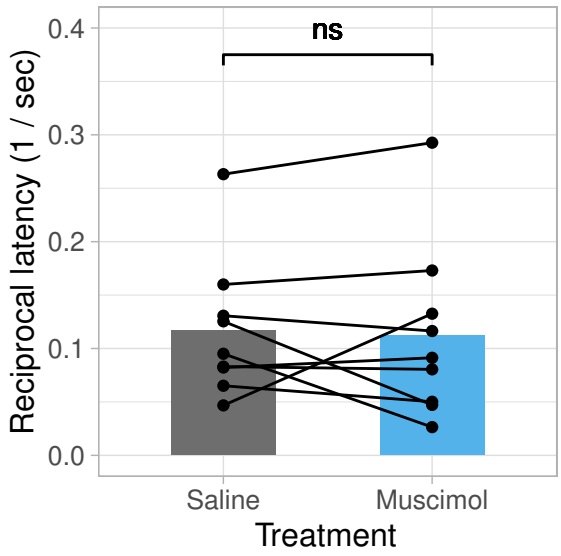


Figure 3.2: Average reciprocal latency as as function of treatment. We found no significant effect of treatment on trial latency using a GLMM ($p > .05$, family = Gamma), indicating that auditory cortical inactivation does not strongly influence the time for mice to leave the nest and choose an arm ($n = 9$).

We found no significant effect of treatment on trial latency using a GLMM ($p > .05$, family = Gamma), indicating that auditory cortical inactivation does not strongly influence the time for mice to leave the nest and choose an arm.

3.3.2 The effect of auditory cortical activity on performance

The effects of muscimol on performance as measured by the auditory and location strategies are presented in figures 3.3 and 3.4. We found that bilateral treatment of muscimol into auditory cortex, significantly decreases performance as measured by the auditory strategy ($p < .05$, GLMM family = binomial), while also significantly increasing performance as measured by the location strategy ($p < .05$, GLMM family = binomial). This demonstrates that bilaterally silencing of auditory cortical activity shifts the strategy the mice use from the auditory back towards the location based one. We also tested these effects using a paired t-test with the individual animal performance and again found a significant decrease in performance as measured by the

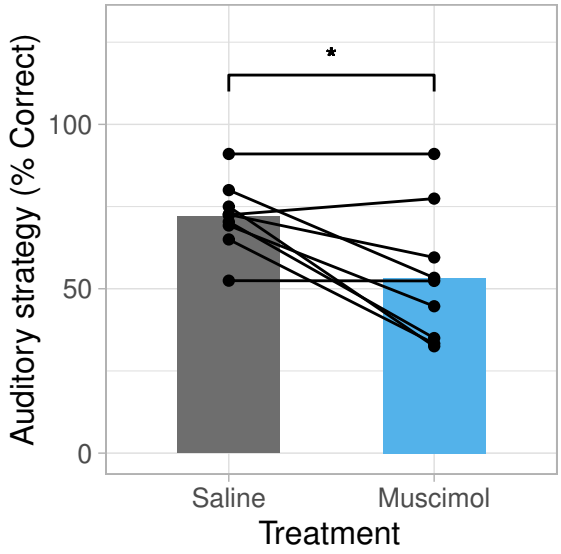


Figure 3.3: The effect of muscimol on % Correct as measured by the auditory strategy for mice that reached criterion. We found a significant effect of treatment on performance as measured by the auditory strategy ($p > .05$, GLMM family = Gamma), indicating that auditory cortical inactivation inhibits mice from using sound to guide them to pups (n = 9).

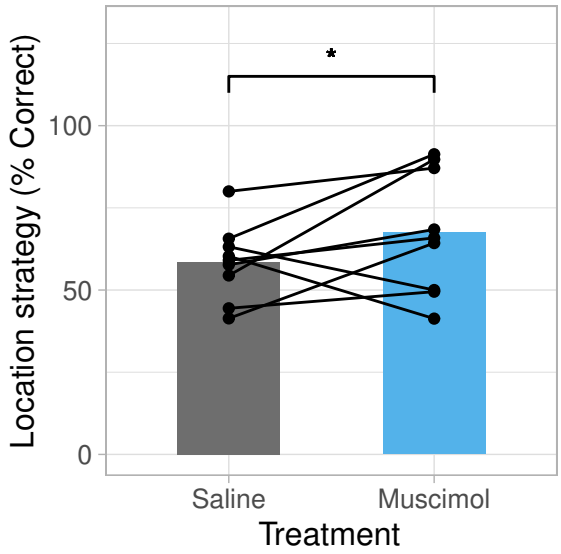


Figure 3.4: The effect of muscimol on % Correct as measured by the location strategy for mice that reached criterion. We found a significant effect of treatment on performance as measured by the location strategy ($p > .05$, GLMM family = Gamma), indicating that auditory cortical inactivation causes mice to revert back to using the location based strategy (n = 9).

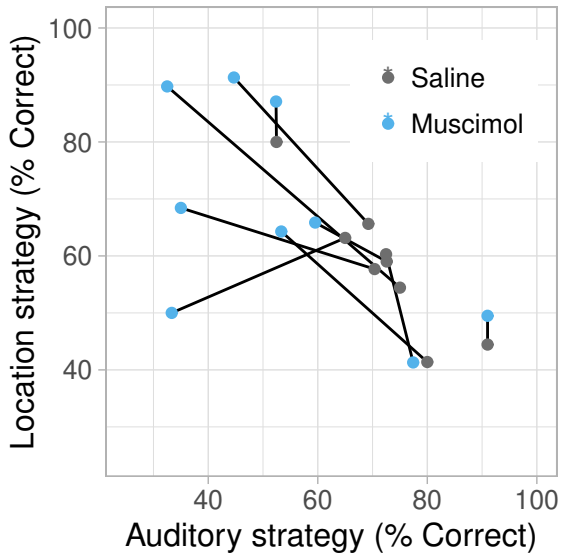


Figure 3.5: When we apply muscimol to auditory cortex before conditioning in trained mice, we see a shift from the lower right to the upper left regions in the strategy space.

auditory-based strategy ($p < .05$), but did not see a significant increase in performance as measured by the location-based strategy ($p > .05$). Indeed the effect of muscimol on increasing the location strategy is smaller than its effect on decreasing the auditory strategy. It would require over 30 mice to have met our learned criteria for the paired t-test to have a power of .8 for the current effect size we see with the location strategy. This highlights the advantage of using the GLMM approach since it increases our statistical power by taking into account the number of samples for each individual mouse.

3.4 Discussion

3.4.1 Relation to prior studies

Prior studies could not resolve whether auditory cortex is or is not involved in our auditory conditioning paradigm. Our data demonstrates it is. Here we discuss this body of literature and highlight the need for our study while describing its relation to prior work.

First, it could not be determined whether auditory cortex is or is not needed for our paradigm, even though our paradigm requires sound localization. It is well established that auditory cortical activity plays an important role in sound localization in non-social contexts. This work was done by reversibly deactivating primary and secondary auditory cortex in the cat with bilateral cooling (Malhotra and Lomber, 2007). However, there are a few reasons why this might not have been the case in our paradigm. The first is that these tasks were significantly harder than the approach task in our paradigm. Sounds were only presented for a brief period of time, on the order of 100ms. Therefore, when the subjects were approaching, no sound was playing. The targets were also spaced 15 degrees apart in the hemisphere. Contrast this with our paradigm where sound is constantly playing during the approach and the target locations are spaced 180 degrees apart. This makes our task much easier, calling in to question whether or not the above results would apply in our paradigm.

Within social context, several studies have eluded to auditory cortical processing as being important, specifically for approach towards infants. Ehret showed the neural processing that is important for preferred approach to pup USVs is lateralized to the left hemisphere of the brain (Ehret, 1987). This finding along with the fact that left auditory cortex has an enhanced representation of ultrasounds (Stiebler, Neulist, Fichtel, and Ehret, 1997), strongly suggests that it is left auditory cortex that is required for preferred approach. However, Ehret never explicitly tested for this and follow up studies focused on auditory cortical activity as measured by cFos expression (Fichtel and Ehret, 1999) as well as the roles of experience and hormonal state (Ehret, Koch, Haack, and Markl, 1987).

A recent study investigating the role of auditory cortical activity in maternal responsiveness was performed by Marlin et al. (2015). They demonstrated that activity within left auditory cortex was necessary for pup retrieval by infusing muscimol via an implanted cannula, very similarly to our treatment group with the exception that

we performed bilateral infusions. At face value it would appear that our study is reproducing these prior results (although being less specific by silencing auditory cortex bilaterally), instead of answering an unknown question. However, after careful inspection, it becomes apparent that this is not the case. The reason for this is that the way this study measured retrieval behavior probably doesn't require auditory information. Retrieval behavior was measured by counting the number of pups retrieved in a short time window (2 min) and did not distinguish between the appetative and consumatory parts of retrieval. The videos from their methods show that although their mice would not retrieve, they would still approach and investigate pups, indicating that the appetative part of retrieval was not impacted. The finding that auditory cortical activity is necessary for retrieval directly contradicts past work that suggests the auditory system is not required for retrieval by deafening rats or silencing their pups (Wiesner and Sheard, 1933; Beach and Jaynes, 1956). This raises the question of which study is correct and indicates that before our study we still didn't know if the auditory cortex was really needed for retrieval.

Our work helps to resolve this contradiction while also providing solid evidence, for the first time, that auditory cortical activity is necessary for a component of retrieval that requires auditory cues. Our mice, with bilateral infusions of muscimol into auditory cortex, still returned pups to the nest when they were presented. This provides further evidence that auditory cortical activity is not necessary for the consumatory part of pup retrieval.

One possible explanation for why Marlin and colleagues saw a retrieval deficit is that they had off target effects from their injection. The amount of muscimol and volume of their injection was substantially higher than values typically reported in the mouse (Letzkus et al., 2011; Banerjee et al., 2017). Regardless, we believe our behavioral paradigm helps resolve these uncertainties by demonstrating that mice can still retrieve when silencing auditory cortical activity and providing an accurate

method to test for auditory task performance in a social conditioning paradigm.

3.4.2 Silence vs silencing

Here we will address a noticeable discrepancy between performance according to the location-based strategy when auditory cortical activity was bilaterally silenced with muscimol, and when our mice were tested on silent trials in the previous chapter (Fig. 2.13). We would like to highlight that the silent trials from the experiment in chapter 2 were dispersed approximately every 5 trials, whereas cortical silencing by muscimol would occur throughout the entire duration of the training. Because of this, it is not possible to distinguish between the difference in location-based strategy performance as being caused by the difference in auditory cortical activity or the difference in trial presentation (dispersed vs sustained). In order to test this we would have to reversibly deactivate auditory cortical activity at the timescale of each individual trial, such as with an optogenetic approach.

3.4.3 Promising paradigm for investigating social auditory learning

Demonstrating that auditory cortical activity is necessary to express the auditory-based strategy in our paradigm suggests that we may be able to use this approach to discover new forms of plasticity that are triggered through auditory social learning. Traditionally, in the maternal mouse model, auditory cortical plasticity has been studied through either anesthetized or awake head-fixed recordings. Auditory cortical responses are different in anesthetized animals but nevertheless plasticity in the representation of auditory cortical responses was found in feed-forward processing (Robert C Liu et al., 2006). Additional forms of plasticity have been discovered through the use of awake head-fixed recordings (F G Lin et al., 2013; Shepard, Lin, et al., 2015). The drawback with these studies is that head-fixed animals can't interact with pups and therefore recordings are done while they are passively listening to sounds.

Several studies have demonstrated that stimulus-elicited sensory responses when animals are actively performing a task, can be different from when they are passively exposed to those same stimuli. For example, receptive field changes can be driven by task engagement (Fritz et al., 2003). It has even been shown that task structure can influence auditory cortical representations (David et al., 2012). These results suggest that to best understand how social experience shapes auditory cortical responses, we want to use paradigms that have mice actively engaged with sound using tasks with as similar structure to the natural behavior as possible.

In summary, our paradigm achieves three important points that make it an ideal candidate for investigating how the representation of sound in auditory cortex changes with social experience. They are

- Produces stereotyped behavior allowing for controlled sound delivery with a high number of trials, which is ideal for characterizing neural responses to sound.
- Allows for social interaction using a natural task structure.
- The task requires auditory cortical activity.

Activity vs plasticity

Several studies have demonstrated plasticity in auditory cortical representations caused by experience in both non-social and social contexts. Pairing sounds with non-social factors such as foot shock (Letzkus et al., 2011) or stimulation of nucleus basalis (M P Kilgard and Merzenich, 1998) can drive plasticity in auditory cortex. Experience depended plasticity has also been demonstrated with motherhood (Rothschild et al., 2013) and social interactions with pups (Banerjee and Liu, 2013). These studies suggest that auditory cortical plasticity may be driven by experience in our behavioral paradigm. A likely mechanism for such plasticity is oxytocin release directly into auditory cortex (Marlin et al., 2015) which has been shown to act through NMDA

receptors to alter tuning for co-occurring sounds (Mitre et al., 2016). One could test whether NMDA receptor mediated auditory cortical plasticity is necessary for this learning in our paradigm using a NMDA receptor antagonist such as AP5. Another approach for blocking plasticity would be to use protein synthesis inhibitors, however these can induce additional confounds that influence neural processing (W. Xiong et al., 2006).

3.4.4 Significance of increase in location-based strategy

The fact that silencing auditory cortical activity improves performance as measured by their location strategy, is informative for how these strategies are represented neurally. This result allows us to rule out encodings where by training, the location-based strategy is effectively over-written by the auditory strategy. This over-writing would occur if synaptic plasticity during learning, altered the neural circuitry responsible for approach in such a way that spatial information could no longer be used to guide the approach. This would mean that silencing auditory cortical activity would cause mice to perform poorly on both trials, similar to the performance on silent trials that are randomly dispersed throughout the training session. Instead, it appears that the auditory strategy is added to the set of possible approach strategies, and is preferentially chosen when the auditory cortical representation is available.

3.4.5 Possible downstream targets

A number of brain areas have been demonstrated to be necessary for the expression of parental behavior in rodents. Of these, the MPOA is likely to be an important nucleus for regulating the behaviors in our conditioning paradigm. The role of MPOA for driving maternal behavior has been well established. Lesions in the MPOA lead to retrieval and nest-building deficits (Numan, 1994) as well as deficits in pup-reinforced instrumental behaviors (A. Lee et al., 2000). Recently, it was discovered that these

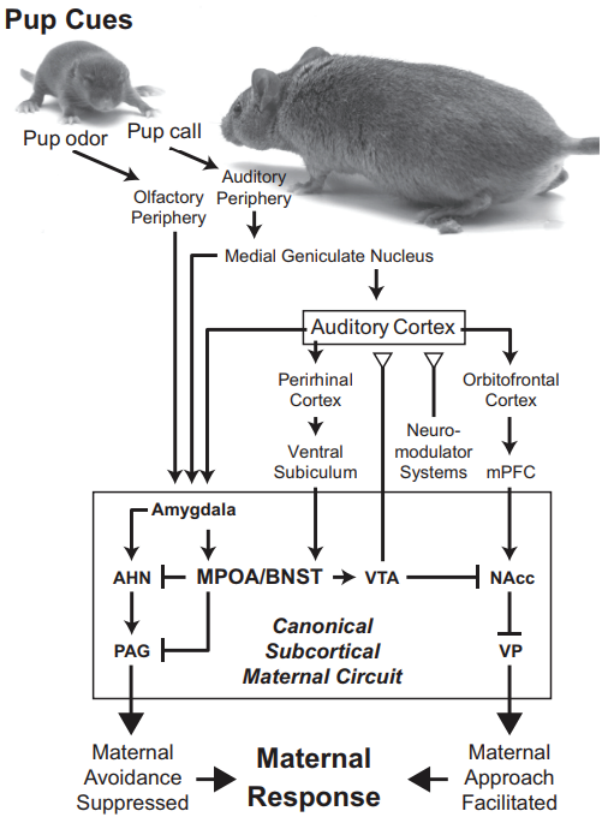


Figure 3.6: Schematic of possible routs for auditory cortical information to influence maternal behavior and the canonical maternal circuit. Small arrows indicate predominantly excitatory connections; bars indicate presumed inhibitory connections; open triangles indicate neuromodulatory inputs. The auditory cortical representation of pup associated cues can be relayed into the maternal circuit through limbic as well as prefrontal pathways. Adapted from Banerjee and Liu (2013)).

behaviors are controlled by a sub population of Galanin expressing neurons within the MPOA (Z. Wu et al., 2014). Therefore, a reasonable hypothesis for how auditory cortical activity comes to influence the approach component of pup retrieval, is by influencing these Galanin expressing MPOA neurons.

The MPOA receives projections from many brain areas. However, no direct projections from auditory cortex to the MPOA have been found. Instead, a majority of its input comes from reciprocal projections from the MPOA itself and the remaining inputs come from 28 different subcortical regions, many of which have previously been identified as playing a role in maternal behavior (Kohl et al., 2018). Although, there

are no direct projections from auditory cortex to the MPOA, there are other possible routes for auditory cortical information to reach the MPOA (Fig. 3.6).

Information from auditory cortex may reach the MPOA through the amygdala. Auditory cortex sends projections to the basolateral amygdala (BLA) which outputs through the medial amygdala (MeA) to the MPOA (Kohl et al., 2017). However, the amygdala to MPOA pathway is typically associated with pup directed aggression and suppressed maternal behaivor. Another possible route into the maternal circuit is through either the perirhinal cortex (Banerjee and Liu, 2013).

It is also possible that auditory cortical activity is influencing retrieval behavior through areas that do not feed into the MPOA. This could be achieved through synaptic plasticity in projections from auditory cortex to the auditory striatum, a mechanism that has been demonstrated in a non-social auditory frequency discrimination task (Q. Xiong et al., 2015). Or through orbitofrontal cortex which could influence circuits downstream of MPOA such as the nucleus accumbens (Banerjee and Liu, 2013). In general, our understanding of how auditory cortical activity is transformed into behavior is quite limited at this point in time, so we also can't rule out the possibility that auditory cortical information is traveling through an unknown pathway. Further studies are needed to identify if auditory cortical activity is influencing pup approach behavior through a pathway that targets the MPOA, or through an alternate route, such as direct projections to auditory striatum, or a yet to be identified pathway.

CHAPTER 4
THE EFFECTS OF MOTHERHOOD ON AUDITORY CONDITIONING

4.1 Introduction

In the previous chapters, we described our behavioral paradigm to pair sound playback with pup retrieval. We saw that naive mice switch from initially having a location-based strategy where they choose to enter the arm where they last received a pup, to an auditory-based strategy where they use the sound to guide their choice for which arm to enter. We also demonstrated that silencing auditory cortical activity with muscimol after mice have learned the auditory-based strategy shifts them back toward using the location-based strategy. In this chapter, we investigate whether motherhood alters the learning process in our behavioral paradigm.

Motherhood induces many physiological changes in the body, which allow the mother to care for and provide nourishment for her baby. These changes begin during pregnancy, and are controlled by reproductive hormones. Key hormones in this process are estrogen, progesterone, prolactin, and oxytocin. Estrogen and progesterone are steroid hormones, which are synthesized and released into the blood stream by the ovaries (Strom, Theodorsson, Ingberg, Isaksson, and Theodorsson, 2012). Prolactin is a peptide hormone, which is synthesized and released into the blood stream by the pituitary gland. Oxytocin is also a peptide hormone, which is synthesized in the hypothalamus but released into the blood stream by the pituitary gland. During pregnancy, estrogen, progesterone, and prolactin serum levels steadily increase, and this increase in concentration signals physical changes, which are necessary for survival of the baby during the peripartum period. The release of estrogen, proges-

terone, and prolactin stops the estrous cycle, prevents further eggs from maturing, prevents uterine contractions, and helps maintain corpus luteum integrity (Soares, 2004). Additionally, their release also drives mammary growth and milk production (Walker, 1972). During parturition, oxytocin release causes uterine contractions.

Motherhood induces more than just physical changes in the body. It is also associated with behavioral changes. One such behavioral change is an increased rate of retrieval for lactating mothers as compared to virgin females. This has been observed in both rats, who typically ignore pups if they are virgins (Dulac et al., 2014), and mice, where although virgin females do not ignore pups, they show retrieval deficits when compared to lactating mothers when tested in a novel environment (Stolzenberg and Rissman, 2011). Rat mothers have also been observed to spend more time on the open arms of an elevated plus maze when compared to virgins (Love et al., 2005). Ostensibly because mothers are more open to taking risks and are more willing to explore and forage for food. Taken together, these changes allow the mother to better ensure her young will survive by increasing her motivation to care for, feed, and protect them (Kinsley et al., 2006).

Not surprisingly, these behavioral changes are also regulated by maternal hormones. For example, receptors for the maternal hormone estrogen are expressed in neurons throughout the brain (Laflamme, Nappi, Drolet, Labrie, and Rivest, 1998). It has been shown that natural variations in maternal care are associated with differences in ERα expression in the medial preoptic area (MPOA) (F. A. Champagne et al., 2003). These receptors would be capable of detecting the elevated levels of estradiol in the blood during pregnancy since it crosses the blood-brain barrier (Banks, 2012). Indeed, nulliparous rats, who would not normally act maternal, exhibit maternal behavior when treated with estradiol implants (Bridges, 1984).

Receptors for the hormone oxytocin are also expressed in neurons in areas that are known to play a role in maternal behavior, such as the bed nucleus of the stria termi-

nalis, the MPOA, and the lateral septum (Francis et al., 2000). Oxytocin is released diffusely throughout the brain from the paraventricular nucleus in the hypothalamus. Changes in brain oxytocin receptor distribution have been associated with the onset of maternal behavior in voles (Insel and Shapiro, 1992), and individual differences in maternal behaviour among rats (Francis et al., 2000). Moreover, intracerebroventricular administration of oxytocin can induce maternal behavior in rats (C A Pedersen and Prange, 1979).

Finally, beyond driving behavioral changes, motherhood can also drive cognitive changes that improve spatial memory (Love et al., 2005; Kinsley et al., 1999). Motherhood is also associated with plasticity in sensory representations (Banerjee and Liu, 2013; Kendrick, Levy, and Keverne, 1992), suggesting the possible enhancement of general associative learning mechanisms, which would improve performance on tasks beyond spatial learning. Indeed, within the context of the maternal mouse communication model, enhanced memory for pup call recognition has been demonstrated in mothers (F G Lin et al., 2013). However, it is unclear if the enhanced spatial learning that is observed in mothers would translate to faster learning in our paradigm, or if it would slow learning in our paradigm, by strengthening the initial spatial strategy of the mice. In this chapter, we tested whether motherhood enhances learning and memory for a novel auditory cue paired with pups through instrumental conditioning using our behavioral paradigm.

4.2 Methods

4.2.1 Experimental design

These experiments used two animal groups: lactating mothers and virgin cocarers. Lactating mothers were group housed with their sisters up until they were mated at around 9-13 weeks of age, at which point they were housed with a single male. Once the mouse was pregnant, approximately 15 days before giving birth the male

was removed from the cage and a cocaring female was introduced to the cage. The cocaring female had previously been group housed with her sisters but was a virgin mouse with no prior experience with males (except for pre-weaning experience with brothers) and no prior experience with pups. Cocaring mice differed from lactating mothers in that they had not undergone pregnancy and presumably the hormonal changes associated with it. Cocaring mice served as a control for pup experience that is not unique to motherhood, such as nest building, grooming, crouching over, and retrieving pups (all maternal behaviors that cocarers do). However, cocaring mice also differed from lactating mothers in some experiences we could not control for, mainly parturition and nursing pups. If a litter was cannibalized, both the mother and the cocarer were removed from the study.

All behavioral studies were conducted inside an 80'-2" × 10'-6" double wall anechoic chamber (IAC, Bronx, NY) under dim red light during the dark phase of the animal's light cycle. Twenty five CBA/CaJ mice (13 lactating mothers and 12 cocarers) between the ages of 12 and 16 weeks were conditioned with our paradigm. Before conditioning, mice underwent 3 days of habituation on our T-maze. Habituation consisted of 15 minute sessions during which the experimenter would stay inside the anechoic chamber, occasionally opening and closing the chamber door every 5 minutes to acclimate the mouse to the experimenter's presence, and to fluctuations in background dB SPL from entering and leaving the testing chamber. After habituation, mice began training using conditioning paradigm 3 for 8 days with the sound stimuli previously described in chapter 2. For each trial, we measured a latency to choose and arm, and performance scores based on both an auditory-based and location-based strategy. Mice were classified as having learned the task if their performance, as measured by their auditory-based strategy over the last two consecutive training days, was significantly greater than chance. For the subset of mice that met this criteria, performance was tested again two weeks later using the same con-

ditioning paradigm in order to test whether the learned behavior degraded over time. During this two week gap, before the next testing session, the litter of a lactating mother was weaned at age p21.

Lactating mothers and cocarers were not conditioned using pups from the litter that they cared for in their home cage. They were conditioned with a set of foster pups from a different litter that was born on the same day as their litter. Over the course of conditioning, to avoid pups growing large enough to crawl on their own around the maze, we replaced the litter used for conditioning with a younger litter. Age ranges for pups during conditioning were all between 3-10 days old. Reciprocal latency and performance was only compared using the subset of mice that met our criteria for having learned the task.

Reciprocal latency and performance were compared between lactating mothers and cocarers using a Generalized Linear Mixed Model (Nelder and Baker, 2004; Wood, 2006) to account for multiple observation per mouse. Observations for both metrics were modeled as being distributed according to an exponential family. The reciprocal latency of each trial was modeled as being gamma distributed, since it is non-negative. To model performance (auditory- or location-based), the score of each trial was modeled as being binomially distributed.

To test for a different rate of reciprocal latency or performance change across training days, we constructed models with 4 fixed effects: an intercept term, a day term, an animal type term, and an interaction term for day and type. Each of these terms were also treated as random effects grouped by each animal. To test for a different rate of performance change across trials, ignoring days, we constructed a different model with 4 fixed effects: an intercept term, a cumulative trial term, an animal type term, and an interaction term for cumulative trial and type. Each of these terms were also treated as random effects grouped by each animal. These models were fit using the lme4 package (Bates et al., 2015) in R (R Core Team, 2017).

4.3 Results

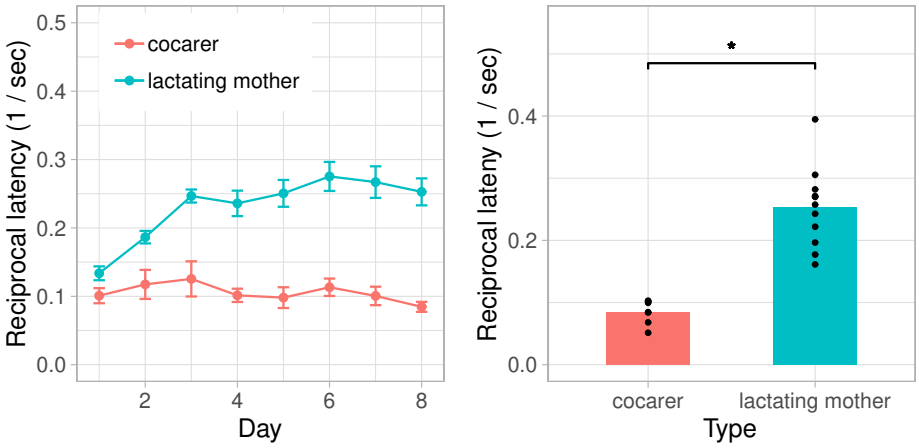
We trained 13 lactating mothers and 12 cocarers. Of these, 11 (84%) of the lactating mothers and 7 (58%) of the cocarers met our criteria for having learned the task. Our analysis were restricted to these subset of mice.

4.3.1 Motherhood increases reciprocal latency

Over training, the reciprocal latency for mothers to approach and enter one of the maze arms on the T-maze increased faster than for cocarers. We found a significant effect of animal type and a significant interaction between animal type and testing day ($p < 0.05$, GLMM family = gamma). For mothers, the greatest changes in reciprocal latency occurred over the first three days (Fig. 4.1a), well before they reached their peak performance on the auditory strategy (see Fig. 4.3). This effect was not seen for cocaring mice, who slightly decreased in reciprocal latency with training ($p < 0.1$, GLMM family = gamma). This demonstrates that cocarers are able to learn without a change in reciprocal latency, a marker for degree of motivation. Average reciprocal latency on the 8th day as a function of animal type is presented in figure 4.1b for mice that learned the task. On days 2-8 we found a significant effect of animal type on trial reciprocal latency ($p < 0.05$, GLMM family = Gamma).

4.3.2 Motherhood accelerates learning

Qualitatively, the learning trajectories in strategy space for lactating mothers and cocarers are similar. Nevertheless, when looking at performance, as measured by the auditory-based strategy, we see a significant fixed effect for animal type ($p < 0.05$, GLMM family = binomial), indicating that mothers learn significantly faster on our task than cocarers. Daily average performance, as measured by the auditory-based strategy, is shown in Fig. 4.3. Since mothers completed more trials per day, we also fit



(a) (b)

Figure 4.1: Motherhood enhances reciprocal latency. (a) We found a significant effect of animal type and a significant interaction between animal type and testing day ($p < 0.05$, GLMM family = gamma) (n = 7 cocarers, 11 lactating mothers). This indicates that over training lactating mothers leave the nest and choose to enter the maze arm faster than cocarers. Error bars represent the standard error of reciprocal latency computed over each animal group. (b) Individual daily averaged reciprocal latency for lactating mothers and cocarers on day 8.

a model which took into account the total number of trials completed to account for their enhanced reciprocal latency. Ten trial average performance, as measured by the auditory strategy, and as a function of the total number of completed trials is shown in Fig. 4.4. Using this model we again found a significant effect of animal type ($p < 0.05$, GLMM family = binomial). This result suggests that motherhood enhances learning in ways that cannot be explained by just the increase in trials completed per day due to the increased reciprocal latency.

4.3.3 Experience alone can create a long-lasting memory

We did not see a significant effect of motherhood on long-term memory for the task when mice were retested 2 weeks after their last training session (Fig. 4.5). The daily averaged performance on the last day of consecutive training and a subsequent training session 2 weeks later were not significantly different (Welch Two Sample t-test). We also fit a GLMM to the auditory-based strategy performance scores

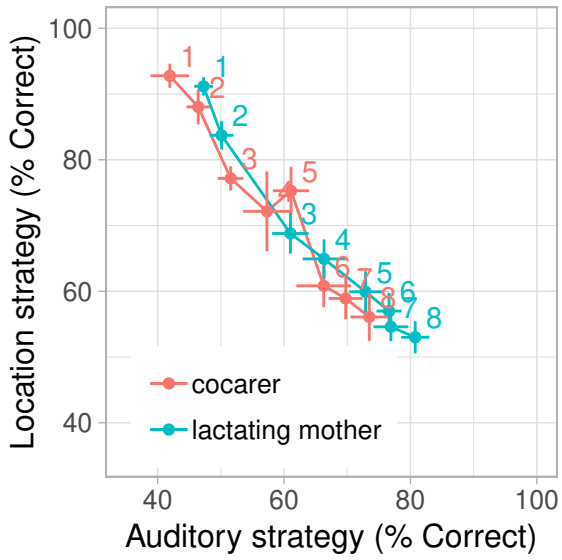


Figure 4.2: Qualitatively, strategy space curves do not differ between cocarers and lactating mothers.

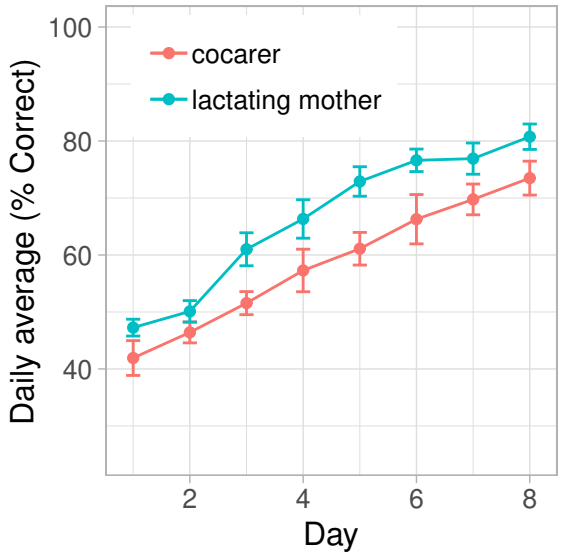


Figure 4.3: Motherhood enhances learning of the auditory strategy. We see a significant effect of animal type on auditory strategy performance ($p < 0.05$, GLMM family = binomial) (n = 7 cocarers, 11 lactating mothers), indicating that mothers learn faster on our task than cocarers.

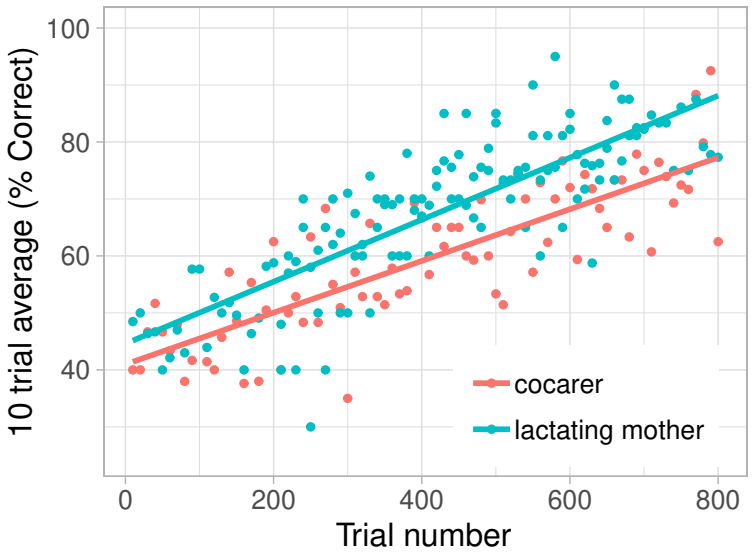
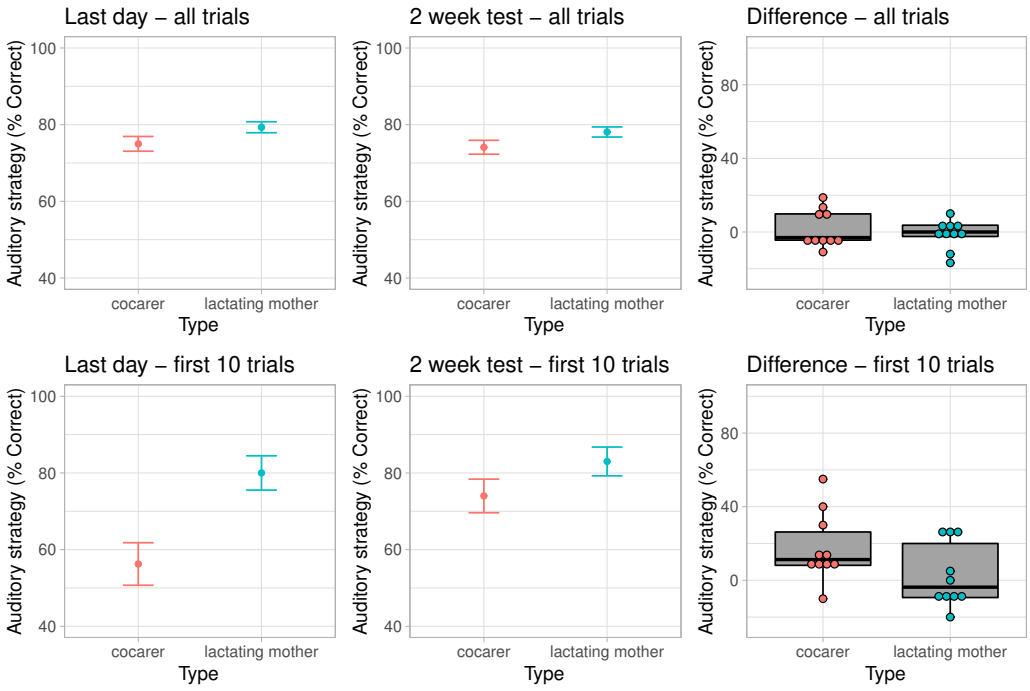


Figure 4.4: Difference in trials performed each day does not explain the accelerated learning in mothers. If we account for the total number of trials each animal completes, we still see a significant effect of animal type on auditory strategy performance ($p < 0.05$, GLMM family = binomial).

for each trial with factors for the animal type and 2 week test. The 2 week test factor was binary and indicated if the trials were being conducted on the last day of consecutive training or the session 2 weeks later. Trials that were conducted before the last day of consecutive training were not included in the model. We expected that performance for cocarers, but not lactating mothers, would drop on the training session 2 weeks later, signifying a decay in memory. This would have resulted in a significant interaction effect between the factors for animal type and 2 week test. Surprisingly, this was not the case. These results hold when comparing performance on all completed trials and when only comparing performance on the first 10 trials wherein any effects of reconditioning should be minimized. Our GLMM did indicate that over these first 10 trials, there was a significant effect of animal type ($p < 0.05$, GLMM family = binomial), with lactating mothers performing better on these early trials than cocarers. However, again there was no significant interaction between animal type and the 2 week test.



(a) (b) (c)

Figure 4.5: Motherhood does not enhance memory for our task. (a) Auditory strategy performance for cocarers and lactating mothers on the last consecutive day of training for all trials and just the first 10 trials of the session. (b) Auditory strategy performance for cocarers and lactating mothers 2 weeks after the last consecutive day of training for all trials and just the first 10 trials of the session. We do not see a drop in cocarer performance as compared to the last consecutive training day. (c) Box plots of the difference between individual animal auditory strategy performance for the 2 week test and last consecutive day of training.

4.4 Discussion

4.4.1 Relation to prior studies

We have presented, for the first time, the effects of motherhood on reciprocal latency, learning, and memory, for a novel auditory cue that has been paired with a pup through operant conditioning. Our results definitively show that motherhood accelerates learning of a novel auditory cue through pup interaction. In this section, we discuss our results in the context of prior work.

Several studies have shown that motherhood increases the prevalence of natural parental behaviors such as retrieving, nest building, and huddling over pups (Dulac et al., 2014; Stolzenberg and Rissman, 2011; Kinsley et al., 2006; Marlin et al., 2015), possibly by increasing the reinforcing value of pups (Alison S Fleming, Korsmit, and Deller, 1994). Our finding that motherhood increased reciprocal latency in our behavioral paradigm is consistent with these studies since reciprocal latency measures the approach component of the natural parental behavior of retrieving, a behavior we already know is increased by motherhood. To the extent that reciprocal latency provides a measure of motivation, our result is also consistent with the interpretation that maternal hormones act on the mother's brain to make them more motivated to work and care for pups (Rilling and Young, 2014).

Information from previous studies could not predict how motherhood would effect the learning trajectory in strategy space. In fact, from those past studies, a case could be made for several possible alternative outcomes on how motherhood could have altered the learning trajectory. The first possibility is that motherhood would strengthen the influence of the underlying circuitry responsible for driving the initial location-based strategy. This is because the initial strategy is based on the pups previous location and it has been shown that motherhood enhances performance on spatial memory tasks (Love et al., 2005; Kinsley et al., 1999). Such an effect could

slow the acquisition of the auditory strategy or block it altogether. This would cause the trajectory to cluster in the upper left region of the strategy space, as we often saw in non-learners. The second possibility is that motherhood could have weakened the innate location-based strategy before they begin to improve their performance on the auditory-based strategy. This could result from mothers being more more exploratory, an outcome that is also supported in the literature (Kinsley et al., 2006). Such an effect would have changed the learning trajectory to curve inward towards the origin. The third possibility (and the one we found), is that motherhood would have increased mechanisms of learning so that they follow an accelerated path down the same trajectory as cocarers. The idea that motherhood improves general learning mechanisms could be supported by a different interpretation of the studies that find enhanced performance on spatial memory tasks (Love et al., 2005; Kinsley et al., 1999), as well as findings from the auditory neuroscience community that motherhood enhances auditory cortical plasticity (Banerjee and Liu, 2013).

Our finding that motherhood accelerates learning, across days by itself may not necessarily be due to enhanced mechanisms of learning, since mothers increased reciprocal latency implies that they complete more trials per day than cocarers. This increase in daily trials could be the cause for what appears to be an enhanced daily learning rate. To account for the possibility that an apparent maternal enhancement in daily learning might just be due to more practice each day, we also compared the learning rate looking at total trials completed, ignoring the effect of day, and still found an enhanced learning rate in mothers. This is significant because it has been known for some time that the ability to recognize pup USVs is accelerated in mothers when compared to cocarers (Günter Ehret, 2005). However there are several interpretations for this result. One is that mothers have an accelerated ability to form the auditory association, and another is that both mothers and cocarers can innately recognize pup calls, but mothers become more motivated to approach them. A large

part of our uncertainty comes from the fact that we don't know if mothers have an enhanced ability to form auditory associations. However, with our result we now know that they do. Although we cannot yet definitively say which interpretation of USV responses is correct, we have provided further evidence to support the hypothesis that mothers learn USVs through an enhanced ability to form auditory associations.

Past work has found that although cocaring mice will recognize pup calls, they lose this recognition after a prolonged period of time not interacting with pups (F G Lin et al., 2013). This differs from mothers who are able to maintain the association after the same time period. We therefore might have expected that in our instrumental conditioning paradigm, we would see a decrease in daily averaged performance for the auditory strategy from the last consecutive training day to the two week retraining day for cocarers but not for mothers. Instead we saw daily averaged performance for both cocarers and lactating mothers remaining constant. We also looked at just the first ten-trial average performance and found instead a slight increase in performance over the two week gap period for both groups. This suggests differences in the stability of the auditory association that is formed when using our instrumental conditioning paradigm versus simply interacting with pups in the home cage, as was done by F G Lin et al. (2013). One possible reason for this could be that our paradigm forms a stronger association over the 8 days of training because mice are continuously retrieving for 50 minutes straight, a condition that likely does not arise often in the home cage. In fact, the average performance we saw by day 8 of training in lactating mothers and cocarers surpassed what is typically reported for preferred approach paradigms (Ehret and Haack, 1981; F G Lin et al., 2013). Another reason we don't see a drop in the auditory-based strategy performance may also be because after two weeks, our mice are being re-tested using a paradigm they already have been conditioned on. The experiments conducted by F G Lin et al. (2013) comparing preferred approach performance in early versus late cocarers were not done within

animal, as a result, the maze was always a new context for the test subject. Therefore, we may be seeing an enhanced memory for our sound cue due to additional contextual conditioning.

Our results from the two week memory test suggest that perhaps cocarers started off worse than lactating mothers on early trials, but their performance within a day improved to look more like lactating mothers on later trials. We decided to investigate this further and found that by plotting performance on just the first or last 50 trials, lactating mothers still outperformed cocarers on both early and late trials for the majority of the training days (Fig. 4.6). Although our data does suggest that had we continued training for longer than 8 days, performance on early trials might have stayed separated while performance on later trials might have converged for our animal groups. This could be interpreted as cocarers "forgetting," so that their performance dropped for early trials on each day, but due to an implicit memory that allows them to be retrained quickly (Schacter, 1987), their performance on later trials was equivalent to lactating mothers. The fact the lactating mothers would not "forget" on early trials does hint at an enhanced form of memory over cocarers, who spend early trials relearning the task. However, future studies would need to be conducted over longer training periods to verify whether this trend plays out.

4.4.2 Possible mechanisms

Possible mechanisms that contribute to the enhanced learning we see in mothers over cocarers are the different concentrations of hormones that coincided with motherhood. The maternal state induces higher levels of estrogen and progesterone for a prolonged period of time relative to the normal fluctuations of the estrus cycle. Motherhood has been linked to enhanced spatial learning (Kinsley et al., 1999; Love et al., 2005), and the altered hormonal state has been shown to increase hippocampal spine density (Kinsley et al., 2006). Although our mice had an innate strategy that was

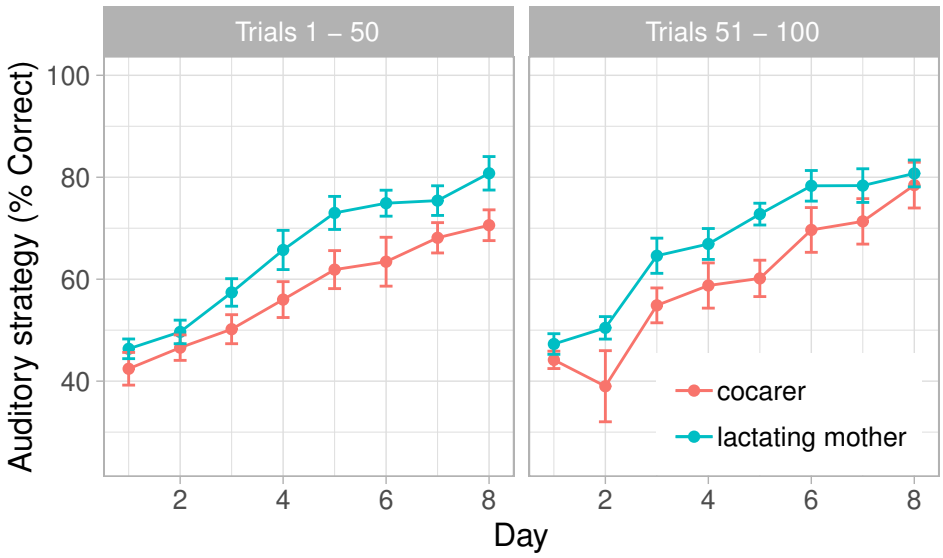


Figure 4.6: Lactating mothers outperform cocarers on early and late trials. On training day 8, for cocarers, performance on early trials is worse than performance on later trials. However, this trend is not present consistently for earlier training days.

spatial, motherhood accelerated the shift away from this spatial strategy when it was inferior to a sensory cue for predicting pup location. This suggests that motherhood could alter learning mechanisms that can be applied to form general sensory associations, possibly through enhanced sensory cortical plasticity. Indeed, sensory cortical plasticity is prevalent in motherhood, with changes in excitatory inhibitory balance (L. Cohen and Mizrahi, 2015), multisensory integration (L. Cohen et al., 2011), and the representation of pup calls (F G Lin et al., 2013; Shepard, Lin, et al., 2015) all having been observed. In line with this, maternal hormones have also been linked to enhanced sensory plasticity and learning. For example, estrogen may act to prime auditory cortex to form a more stable encoding of sensory cues (Banerjee and Liu, 2013), and has been shown to enhance the recognition of pup calls in mothers (Koch and Ehret, 1989). Another likely hormonal source for enhanced sensory learning in mothers is oxytocin. Auditory cortex receives oxytocin projections and its release has been shown to drive plasticity for sounds that were recently heard (Mitre et al., 2016).

CHAPTER 5
CONCLUSION

5.1 Summary

By interacting with pups in their home cage, female mice "learn" to approach the ultrasonic vocalizations emitted by pups. Hence, when tested with distant speakers playing back ultrasonic models of pup calls, they prefer to approach these over playback of other sounds with different acoustic properties, presumably because they recognize the calls as behaviorally relevant. This preference does not occur in naive virgin mice that have not had pup rearing experience, and takes several days to emerge in cocaring animals, while the process is accelerated in mothers (Günter Ehret, 2005). Additionally, auditory cortical plasticity is observed in mice that have acquired a preferred approach to pup calls, which presumably serves to enhance the detection and discrimination of pup sound cues (Robert C Liu and Schreiner, 2007; Shepard, Lin, et al., 2015; Galindo-Leon et al., 2009). This experience-dependent plasticity has traditionally been considered to be driven by general mechanisms of learning (i.e. will enhance the representation of sounds that become meaningful through the experience). This idea is supported by recent work showing that oxytocin release into auditory cortex can shift neural tuning curves for tones that are paired with the release (Mitre et al., 2016). On the other hand, recent studies highlight innate circuitry for parenting that does not require learning to express. For example, maternal responsiveness to pups can be initiated by the optogenetic activation of a galanin-expressing subpopulation of neurons within the medial preoptic area (MPOA). The corresponding parental response just requires the activation of the right latent neural circuit without learning. This raises the question of whether or not the auditory

cortical plasticity that has been observed was caused by the unlocking of a latent circuit, rather than the expression of general learning mechanisms. A major challenge in answering this question is that with previous paradigms, the social interactions with pups that lead to preferred approach and auditory cortical plasticity, have been treated like a black-box.

In chapter 2, we wanted to address this by finding a behavioral training paradigm that could serve as a model for how socially interacting with pups in the home-cage could form an association for acoustic pup cues. Specifically, we asked if a novel sound under experimenter control could be paired with a pup. We wanted such a behavioral paradigm to satisfy two constraints. We needed the mice to complete at least 100 trials in under an hour to allow for neural characterization. We also needed the mice to exhibit a stereotyped behavior that would allow for the repeated delivery of sound from a fixed orientation relative to the mouse's head for each trial. We considered the retrieval component of parental behavior and found that continual delivery of pups at the end of a linear approach track produced a steady, stereotyped retrieval of over 100 pups in a 40 min time period with no indication of stopping. We took advantage of this robust retrieval behavior and developed a paradigm to pair sound with a pup that satisfied our constraints by guiding the approach component of retrieving with an auditory cue delivered from one of two speakers placed at the top of a T-maze. We found that after 7 days of training, most of our virgin mouse population met our criteria for learners. We also found that for early training days, when task performance was at chance, our mice had an initial location-based strategy where they entered the last arm they received a pup.

After developing this behavioral paradigm, we recognized that there was considerable uncertainty in the literature regarding whether or not auditory cortical activity would be needed for our task. While some auditory conditioning paradigms require auditory cortical activity and plasticity (Banerjee and Liu, 2013), the conditions

for which auditory cortical function is needed even in standard auditory conditioning tasks is not well understood (LeDoux et al., 1991; Campeau and Davis, 1995). It is possible that our amplitude-modulated broadband noise stimulus may not be "complex" enough to engage auditory cortex. There are subcortical projections from auditory thalamus that would allow for information to bypass auditory cortex. Auditory thalamus sends direct projections to basolateral amygdala and perirhinal cortex (LeDoux et al., 1991; Campeau and Davis, 1995), brain areas that are part of the pathways for both pup directed aggression and pro-parental behavior (Dulac et al., 2014; Banerjee and Liu, 2013; Simerly and Swanson, 1986). Because of these reasons, we could not simply assume that our conditioning paradigm would require auditory cortical activity.

In chapter 3, we tested the effect of auditory cortical activity on motivation and approach strategy in our paradigm. We bilaterally silenced auditory cortex with muscimol in naive mice after training to criterion on the auditory-based strategy. We found no significant effect of treatment on reciprocal latency, indicating that auditory cortical inactivation does not strongly alter motivation. However, bilateral treatment of muscimol into auditory cortex significantly decreased performance as measured by the trained auditory-based strategy, while also significantly increasing reliance on a location-based strategy. This demonstrates that bilaterally silencing auditory cortical activity shifts the strategy the mice use from the trained back towards their initial one.

After demonstrating that auditory cortical representations are being used to direct the auditory-based strategy, we next asked whether motherhood could impact learning in our paradigm. Motherhood induces many physiological, behavioral, and cognitive changes that facilitate improved maternal care. Key changes in mothers that could alter how they learn when compared to virgin cocaring mice in our conditioning paradigm are their increased prevalence for taking risks (Love et al., 2005), increased

spatial memory (Kinsley et al., 1999), and increased sensory plasticity (Banerjee and Liu, 2013; Kendrick et al., 1992). However, it is unclear whether these differences mothers could translate to faster sensory learning.

In chapter 4 we tested this by comparing reciprocal latency, learning rate, and memory between lactating mothers and cocarers in our auditory instrumental conditioning paradigm. We found that over training, the reciprocal latency for mothers to approach and enter one of the maze arms on the T-maze increased faster than cocarers. We found a significant effect of animal type and a significant interaction between animal type and testing day. Qualitatively, we did not see a large change in the trajectories in the strategy space for lactating mothers and cocarers, suggesting that both animal groups take the same "strategic" approach to learn the task. When looking at performance, as measured by the auditory-based strategy, we saw that mothers learned significantly faster on our task than cocarers even after accounting for the greater number of trials completed daily by mothers. This suggests that motherhood enhances learning in ways that cannot be explained by just an increase in motivation to act maternally. Finally, we did not see a significant effect of motherhood on long-term memory for the task when retested 2 weeks later. This was a surprising result, indicating that cocarer performance does not decrease when retrained after a 2 week period without experience on the maze.

5.2 Future directions

The conditioning paradigm we have developed in this thesis opens the door to investigating many exciting questions we in the social auditory neuroscience community would like to pursue. Here we discuss those that are most immediately relevant in order to be a guide for future work.

The most obvious next step for our behavioral paradigm is to investigate whether the plasticity that has been observed in mothers for pup USVs is also observed for

the novel sound conditioned on our paradigm. The spectral energy of natural pup USVs is concentrated within 60-80 kHz. Our lab has previously shown that mothers have significantly stronger pup call single unit inhibition at recording sites where the LFP is tuned to a frequency (< 50 kHz) that is lateral to the natural pup call range (Galindo-Leon et al., 2009). This mechanism putatively enhances the contrast of neural population activity between neurons responsible for representing salient calls and neurons responsible for representing other stimuli. The energy of our auditory conditioning stimulus is concentrated within 25-55 kHz. Therefore, if the lateral band inhibition we observed in mothers for USVs was due to associative learning, we predict that when mothers are conditioned to approach our novel sound for pups, we would observe stronger single unit inhibition in response to the auditory conditioning stimulus at recording sites where the LFP is tuned to a frequency greater than 55 kHz, which is lateral to the conditioning stimulus range. This question could be investigated with head-fixed recordings comparing animals that have been conditioned to approach our novel stimulus to animals that have been just passively exposed to the sound.

By recording from ensembles of neurons using multi-channel probes, it is possible to simultaneously record from auditory cortex, other sensory areas, and nodes within the canonical maternal circuit such as the MPOA. Such studies would allow us to better understand how auditory cortical representations interact with other sensory systems and how these representations drive subcortical activity in the maternal circuit. With the advances in multi-channel silicon probes, the future looks bright for labs that seek to record representations of ensembles of neurons across cortical and subcortical brain areas. Furthermore, freely-moving recordings during behavior could investigate how these representations change as the association with pups is formed. Key points during which we would want to characterize auditory cortical activity include halfway down the T-maze arm when the mouse's behavior is most

stereotyped; at the decision point of the T-maze; and at the ends of the T when the mouse either does or does not receive a pup. Such studies would reveal to what extent sounds are modulated by social experience, reward, and expectation.

There are lots of questions we could ask by manipulating what sound the animals learn. One line of questioning has to do with whether all sounds are able to be learned equally well, for example due to evolutionary predispositions (Morton, 1977). This could be tested by first training mice on the auditory stimulus used in this dissertation, and once they reach criterion performance, switch the stimulus to some new sound with different acoustic properties (narrower or wider bandwidth, has frequency sweep structure). This would serve as a within animal test to compare the time it takes to learn these different sounds, allowing for the exploration of how various acoustic properties impact sound association learning in a social context. Another line of questioning has to do with the specificity of the auditory learning. Behavioral studies using multiple sounds would allow for the investigation of more traditional auditory attention questions within a social context. Specifically, including distractor sounds during training could test the specificity of the learned association and probe the auditory system's ability to discriminate across various acoustic dimensions.

Another extension to our behavioral paradigm would be to develop a non-social control. This would be a way to pair sounds with a behavior that is similar to pup retrieval, but does not utilize social interaction. One possible approach would be to use water or food reward instead of pups. We attempted this using water reward but ultimately were not able to get a stereotyped behavior from our mice at a suitably high rate due to the inability of the animals to naturally return to the nest. If successfully developed though, such a non-social task with the same motor elements during approach as our social conditioning paradigm could allow for exciting experiments to be conducted. Specifically, we could ask whether social neuromodulators like estrogen or oxytocin enhance auditory learning only in social

contexts.

Even without a non-social control though, future studies could still use our paradigm to test if social neuromodulators like estrogen or oxytocin enhance auditory learning when a sound is paired with pup reward. This would be achieved by bilaterally cannulating auditory cortex, similar to our approach in chapter 3, and infusing a social neuromodulator before training everyday. Similarly, to test for necessity, antagonists for these neuromodulators could be infused before training. Additionally, the experiment in chapter 3 could be extended to silencing auditory cortical activity after training to investigate its role in consolidation of social sounds. For example, oxytocin receptors are more concentrated in left auditory cortex than right auditory cortex, and it is the activity specifically in left auditory cortex that is necessary to drive maternal behavior (Marlin et al., 2015). Our silencing experiment in chapter 3 was performed bilaterally, so a natural next question could be to test whether left auditory cortical activity is more important than right auditory cortical activity in learning to approach or in expressing a learned approach for our novel sound stimulus when paired with pups.

Another point we would like to highlight is that we have demonstrated auditory cortical "activity" is needed to express the learned approach to sound for pups. We have not shown that during training, changes in the auditory cortical representation, or auditory cortical "plasticity", is also needed for mice to be able to learn to approach sound for pup reward. One mechanism of auditory cortical plasticity that has been shown to be driven by oxytocin release is NMDA receptor mediated synaptic plasticity (Mitre et al., 2016). Therefore, one possible experiment to test if auditory cortical plasticity is needed in our paradigm is to infuse the NMDA channel receptor antagonist AP5 bilaterally into auditory cortex daily before conditioning sessions and test if that blocks or delays learning.

Optogenetics could be used to address questions about the specific temporal win-

dow when neural activity in key brain areas during our conditioning paradigm is needed. We found in chapter 3 that when silencing auditory cortical activity with muscimol, mice show a shift back towards their initial location based strategy. However, in chapter 2 we did not see this same shift for silent trials that were randomly dispersed among 20% of all trials on day 8. This suggests an experiment where we optogenetically silence auditory cortex only for various percentages of trials and test if the percentage of silent trials significantly effects whether or not the mice shift their strategy back to being location based. Optogenetic stimulation of subcortical key nodes in the canoncial maternal circuit has been shown to enhance pup retrieval and maternal motivation (Kohl et al., 2018). This suggests studies where we optogenetically stimulate these key nodes during conditioning on our paradigm in virgin mice. Such experiments would explore if activation of these brain areas are sufficient to explain the enhanced learning rate we see in mothers.

There are several other future approaches worth pursuing we would like to mention. The first approach is to address the fact that we really do not know how different the hormonal state of a mother is from a cocarer. It has been shown that sustained experience with pups elicits parental behavior, but whether this onset in parental behavior alters the hormonal profile of the animal as dramatically as in a mother is not well understood. Presumably pup are attempting to nurse from the cocarer and these interactions quite possibly could cause oxytocin release. This could potentially be investigated with microdialysis study. Additionally, we would like to mention that future pharmacologic manipulations could benefit from using DREADDs technology instead of cannulation. The superficial location of auditory cortex makes it especially susceptible to cannula damage, which may have contributed to the low percentage of mice that successfully reach criterion in our muscimol study in chapter 3. Future work could also utilize tracer studies to identify the path by which auditory cortical information influences parental behavior.

This dissertation demonstrates for the first time, a robust paradigm to "pair" a novel auditory stimuli with pups, and that the expressed association depends on auditory cortical activity. This paradigm looks to be a valuable tool for investigating how auditory cortical representations can influence behavior in a social context. It will allow for the investigation of the function and biological mechanisms underlying plasticity for sounds that acquire meaning within a social context, ultimately achieving a method to study a sensory-specific component to social information processing and learning.

Appendices

APPENDIX A

EXPERIMENTAL ANIMALS

Table A.1: Chapter 2 Animals

	Animal	Type
1	E315021402A	virgin
2	E315021403A	virgin
3	E315021404A	virgin
4	E415042806A	virgin
5	E415042807A	virgin
6	E416040802B	virgin
7	E416040803B	virgin
8	E816040802A	virgin
9	E816040803A	virgin
10	E817052802A	virgin
11	E817052804A	virgin
12	E817052803A	virgin
13	E917062002A	virgin
14	E917062003A	virgin
15	E917062004A	virgin
16	E1017101402A	virgin
17	E1017101403A	virgin
18	E1017101404A	virgin

Table A.2: Chapter 3 Animals

	Animal	Type
1	E717062702A	virgin
2	E717062703A	virgin
3	E717071004A	virgin
4	E717071005A	virgin
5	E717072803A	virgin
6	E717072805A	virgin
7	E717072804A	virgin
8	E918020402A	virgin
9	E918020503A	virgin
10	E918020504A	virgin
11	E918021404A	virgin
12	E918021405A	virgin
13	E918021403A	virgin
14	E1018041201A	virgin
15	E1018041202A	virgin
16	E1018041203A	virgin
17	E1018041204A	virgin

Table A.3: Chapter 4 Animals

	Animal	Type
1	E616091904A	cocarer
2	E616091903B	cocarer
3	E816101302A	cocarer
4	E816101306A	cocarer
5	E616113004C	cocarer
6	E616113002C	cocarer
7	E617010604A	cocarer
8	E617010602A	cocarer
9	E917021103A	cocarer
10	E917021104A	cocarer
11	E617032702A	cocarer
12	E617032704A	cocarer
13	E416070105A	mother
14	E416070605A	mother
15	E416072002B	mother
16	E1016081401A	mother
17	E716082205A	mother
18	E516090701B	mother
19	E516090901A	mother
20	E616091903A	mother
21	E816101301A	mother
22	E816101305A	mother
23	E616113003C	mother
24	E617010601A	mother
25	E917021101A	mother

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